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Biochemical Studies of Expired Air in Relation to Ventilation.

DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF PURE SCIENCE OF COLUMBIA UNIVERSITY

BY

CHARLES WEISMAN, M.S.

NEW YORK CITY.

1913

EASTON, PA.:
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May, 1913.

Biochemical Studies of Expired Air in Relation to Ventilation.

INTRODUCTION

In 1911, Rosenau and Amoss[1] published the results of anaphylactic experiments which, in their belief, demonstrated the presence of volatile protein in the breath. The work consisted in condensing the exhalations from the lungs, injecting the liquid thus obtained into guinea pigs and, after a suitable incubation period of two weeks or more, testing the guinea pigs to see if they had become sensitized by the injected condensed breath. Since the guinea pigs reacted to the injections, Rosenau and Amoss concluded that the animals were sensitized with a substance of human origin, protein in character, and further that the protein was volatile in nature.

As the anaphylaxis reaction is extremely delicate, revealing the presence of so minute a quantity as one millionth of a cubic centimeter of blood serum, it is much more delicate than any chemical test. Further, the anaphylaxis reaction is of special value because it indicates a specific sensitizing substance.

The fact that organic matter is present in the expired breath does not necessarily mean that this is toxic. Yet, as pointed out by the editor of the *Journal of the American Medical Association*,[37] the mere demonstration of the presence of such matter is an advance on our present imperfect knowledge of the action of impure air. Further, as Rosenau and Amoss state, this finding would also solve the problem of the anaphylactic reaction sometimes observed after the first injection of foreign serum, particularly in diphtheria antitoxin. And finally, it would serve as the first instance of a phenomenon hitherto unsuspected, the volatilization of a substance having the complex composition and high molecular weight of a protein. In view of the important significance of Rosenau and Amoss' announcement, it was concluded to repeat this work and apply it to problems in ventilation.

HISTORICAL PART.

A CHRONOLOGICAL SUMMARY.

It has long been observed that whenever large numbers of people gather within a confined space, the air becomes vitiated; for susceptible people, a sojourn in such an atmosphere is followed by more or less pronounced symptoms of headache, dizziness, nausea or faintness, etc. This phenomenon has long attracted the attention of scientists.

Many theories have been advanced in explanation of the symptoms observed. The ancients, including Aristotle, had no knowledge of the chemistry of the air. They generally attributed the death of animals confined in a closed space to the warming of the air. In the middle ages this subject received very little attention.

In 1674 Mayow announced that there was a "principle" existing in air capable of supporting life and combustion. Boyle, in 1680, showed that air must be constantly renewed to maintain life. Veratti in 1750 raised the question as to whether death in a confined space was caused by the diminution of the elasticity of the air or by a poisonous exhalation, which, leaving the body of the animal through respiration, carried with it a noxious principle, or by the destruction of the vital principle of the air. With reference to exhalations from the body, Bergman, professor of chemistry in the University of Upsala, writing in 1791, states: "The lungs moreover add by their constant motion, various subtle particles by means of the absorbing vessels, and again by the exhaling vessels, carry off others."

The investigations begun by Mayow, Boyle and Black, and extended by Scheele, Cavendish and Priestley, culminated in the brilliant researches of Lavoisier. Writing of the effects of vitiation of air upon animals, Lavoisier states: "If one encloses animals in a given quantity of air, they perish in it when they have absorbed or converted into carbon dioxide the greater part of the respirable portions of the air. To make respirable the vitiated air, two things must be done: First, to remove from this air by the lime or caustic alkali, the portion of carbon dioxide that it encloses; second, to return to it a quantity of air that is eminently respirable equal to that which it has lost." These conclusions of Lavoisier have lost nothing of their value to-day.

Other investigators sought a cause of air contamination other than carbon dioxide. As early as 1802 we find a reference to this elusive substance. William Barnwell,[2] surgeon in the employ of the East India Company of London, says: "As for an eudiometer or any other apparatus, discovering different effluviae of vegetable, or animal origin, it is probable that we might as well expect to discover it by mechanical or by any pneumatic instruments, ... yet we are very sensible of effluvia in the air, which no instrument, other than the olfactory nerves can discover."

Robertson,[57] in 1808, after discussing the accumulation of carbon dioxide in living rooms and the necessity for air renewal also refers to this unknown substance. "Dr. Guthrie found, that the moisture frozen on the insides of the windows of Petersburgh, during winter, is highly volatile; and on being thawed, it gives out a gas equally pernicious, and in other respects possessing similar properties to those of carbonic acid gas. In a matter of so much importance, an attention to keep a free current of air in places where people pass the greatest part of their time, cannot be too earnestly inculcated."

With regard to the effect of certain mixtures of oxygen and carbon dioxide, Robertson states that Dr. Powell immersed "a full-grown bird in a mixture of three-eighths of oxygenous and five-eighths of carbonic acid gas, and he survived but a few seconds."

The first stimulus for a scientific treatment of the ventilation problem appears to have been given as a result of a report by Peclet[47] on the construction of a prison in Paris. From Peclet's report it is seen that as early as 1843 to 1845, the carbon dioxide content of the air had been used as a measure for the efficiency of ventilation.

Leblanc,[38] in 1842, tried the effect on animals, of breathing large quantities of carbon dioxide. He placed a dog, a guinea-pig, a bird and a frog in a closed room having a capacity of 22.5 cubic meters and supplied it with a large quantity of carbon dioxide gas. At the end of seven minutes the dog was uneasy; at the end of fifteen minutes he suffered more; at the end of twenty-five minutes the candle went out; and finally, after three-quarters of an hour, the bird and dog were in agony, the guinea pig was suffering, and the frog had swollen enormously. The air collected at this time contained 30.4 per cent. of carbonic acid and 16.01 per cent. of oxygen. Notwithstanding this large amount of carbon dioxide, the animals gradually recovered on exposure to fresh air. Leblanc also showed that under the conditions in which the quantity of carbon dioxide rises perceptibly in confined places where crowds gather, the reduction of oxygen is quite insignificant, the proportion rarely falling below 20 per cent. Regarding the increase in carbon dioxide in such places he stated that "when the proportion of carbon dioxide contains 1 per cent. from the effects of respiration, the sojourn of persons in such an atmosphere cannot be prolonged without soon arousing a sensation of pronounced discomfort. There seems to be no doubt that the mere presence of carbon dioxide in that quantity, in confined places can exercise a harmful influence on the organism, above all, if that action is prolonged for some time. One-half per cent. of carbon dioxide, accumulated in a confined space is a limit that should never be exceeded." Referring to the presence of matter of animal origin in the air he states that it is demonstrable only by the odor and adds: "We will probably have to get help from some science other than chemistry to shed some light on these phenomena that are still so obscure."

In the course of a study on the effect of different gaseous mixtures on the vitality of animals, Regnault and Reiset,[52] in 1849, confined a dog in an atmosphere containing 53 per cent. of oxygen. At the end of 22 hours the animal was very uneasy but did not appear to suffer. Analysis of the air showed 17 per cent. carbon dioxide and 40 per cent. of oxygen. They concluded that an animal may live for a long time in an atmosphere enclosing more than half of its volume of carbon dioxide without feeling any apparent discomfort, provided that the atmosphere contains a sufficient amount of oxygen.

Gavarret,[23] in 1851, reported on the poisonous properties of organic emanations of animal origin. He subjected animals to an atmosphere contaminated with animal exhalations and though he replaced the oxygen as fast as it was consumed and removed the carbon dioxide as fast as it was formed, he found that the animals died.

Claude Bernard,[5] in 1857, studied the effects of different atmospheric gas mixtures on animal life. To determine the minimum amount of oxygen necessary to sustain life, he placed a rabbit in a 12 liter bell-jar, and removed the products of respiration by connecting the jar, through a series of U tubes containing sulphuric acid and pumice stone, Liebig potash tubes and calcium chloride tubes, with an exhaust pump. The animal died when the proportion of oxygen had decreased from 21 per cent. to 3–5 per cent.

Bernard also tried the effect of allowing an animal to consume the oxygen in the confined space without removing the carbon dioxide. After exposing a sparrow, weighing about 13 grams, in a 2 liter bell-jar for three hours, without withdrawing the products of respiration, the animal was nearly dead; it still had sufficient life to be able to fly when it was removed from this atmosphere and warmed. The air in the bell-jar contained 3.5 per cent. oxygen and 17.5 per cent. carbon dioxide. When at the end of the second hour of confinement in the bell-jar, the sparrow just mentioned was still lively, a second and later a third sparrow were placed in the jar. The newly introduced sparrow succumbed almost at once. When the sparrow that was removed at the end of the third hour and which had regained its strength was again introduced into the jar it died at once. This showed that when an animal is subjected to an environment which gradually deteriorates, a morbid condition will be gradually produced with a depression of its functions, as a result of which a sort of equilibrium will be established between it and its environment.

On trying the effect of an atmosphere richer in oxygen than atmospheric air, Bernard found that a bird died in $2^1/_2$ hours in an atmosphere that then contained 39 per cent. of oxygen and 13 per cent. of CO_2. An atmosphere of equal parts of oxygen and nitrogen had no effect on animals, but one containing equal parts of oxygen and carbon dioxide caused immediate death. He explains the toxicity of the carbonic acid when respired to be due to the fact that it deprives the animal of oxygen. Bernard also showed that carbonic acid is not poisonous when injected into animals subcutaneously or intravascularly.

Results similar to those obtained by Bernard were reported by Valentin[67] and Bert.[6] Valentin, however, attributes the toxicity of carbonic acid when inspired in an atmosphere containing a greater percentage of oxygen than in the ordinary atmosphere, as due directly to the carbon dioxide itself and not to the fact that the presence of carbon dioxide deprived the animal of oxygen.

Müller,[44] in 1858, found that his animals began to breathe with difficulty when the oxygen went down to from 5 to $7^1/_2$ per cent. while at 15 per cent. there were no changes observable. When he allowed animals to breathe in an atmosphere of oxygen until they died he found[45] at the end of the experiment from 20 to 68 per cent. of CO_2.

Richardson, in 1861,[56] investigated the effect of changes of temperature on the life of animals confined in an enclosed space. He found that a temperature much over or under 20° C. shortened the lives of the animals considerably, especially, if an atmosphere rich in oxygen was employed. In the latter case after removing the carbon dioxide formed and passing an electric spark through the confined air, the devitalized air was again rendered respirable.

Pettenkofer, in 1860–63,[49] showed that the effects of vitiated air in confined spaces were not due to an excess of carbon dioxide or to a deficiency of oxygen in the air; neither of these in the proportions ordinarily found is sufficient to produce harmful results. He did not believe that air contaminated by breathing became a poison in the ordinary sense of the term, but that the harmful effects of such air is due to the diminished power of resistance of those living in such an atmosphere for some time. He emphasized the idea suggested by Leblanc in 1842, that the proportion of carbon dioxide in the atmosphere of inhabited places affords a true index to the amount of other impurities resulting from the respiratory and other exhalations in the confined space.

Hammond, in 1863,[27] reported an experiment somewhat similar to the one of Gavarret.[23] A mouse was confined in a large jar, in which were suspended several sponges saturated with baryta-water. By this means the carbonic acid was removed as fast as formed; this was proven by the fact that when a portion of the air in the bell-glass was passed through a solution of barium hydroxide, no barium carbonate, beyond a very small quantity, was formed. Fresh air was supplied through a U tube connected with the bell-glass and closed by a valve which was formed by a little water in the bend of the tube. As the air in the bell-glass was rarefied by respiration and the absorption of the carbonic acid, fresh air flowed in from without, while the arrangement of the tube prevented the air of the bell-glass from passing out. The water vapor exhaled by the animal was absorbed by two or three small pieces of calcium chloride.

Under the above conditions the mouse died in 45 minutes. The observation was repeated many times and death invariably ensued in less than an hour. When the vitiated air was passed through a solution of potassium permanganate, the presence of organic matter in large quantities was demonstrated.

As a result of this work, Hammond believed that the organic emanations from the bodies of man and other animals in a condition of comparative health are positively noxious. That the organic exhalations from the lungs contain organic matter, was demonstrated by causing expired air to pass through, pure, colorless, sulphuric acid. As a result of the carbonization of the organic particles the sulphuric acid became of a very perceptible brown color. The presence of organic matter was also demonstrated by its reaction with potassium permanganate and by the fact that the condensation water from this air readily underwent putrefaction.

Hammond also studied the effects of artificial mixtures of oxygen and carbon dioxide. He criticized Bernard's[5] work on this subject claiming that the death of Bernard's experimental animal was due to the fact that his animal was placed in a mixture of carbon dioxide and oxygen which was contaminated by exhalations from the other animals that had been previously placed in the bell-jar. Hammond confined a sparrow under a large bell-jar having two openings. Through one of these he introduced, every hour, 1000 cubic inches of an atmosphere containing 45 parts of oxygen, 30 parts of nitrogen and 25 parts of carbon dioxide, allowing the vitiated air in which the animal had respired partially to escape. At the end of twelve hours the bird was in as good a condition as at the commencement of the experiment. A mouse subjected to a similar experiment also suffered no inconvenience. From his experiments he was led to believe that carbon dioxide is not positively poisonous, but only negatively so, when its presence is unaccompanied by a due amount of oxygen. When this latter gas is present in the proportion of two parts to one of CO_2, life can be sustained.

Hammond also injected carbonic acid gas into the cellular tissue of rabbits and dogs without the least injurious result and even introduced it, with impunity, directly into the blood. In spite of these findings he believed that an amount of carbonic acid in the atmosphere above the normal quantity is harmful in effect, depending upon the extent to which it is present, inasmuch as it prevents the absorption of a corresponding amount of oxygen.

Ransome,[50] in 1870, investigated the amount of organic matter in the breath. The aqueous vapor of the breath was condensed in a large glass flask surrounded by a freezing mixture. It was found that in ordinary respiration about 0.2 gram of organic matter is given off from a man's lungs in twenty-four hours. Cells of various kinds, portions of epithelia, etc., were found in the breath. This author also called attention to the necessity for ventilation.

R. Angus Smith,[64] in 1872, studied the physiological effects of carbonic acid in its relation to ventilation. A man was subjected to the atmosphere of an air-tight lead chamber 6 feet long, about 4 feet wide and 8 feet broad, for a number of hours. After four hours the pulse went down from 73 to 57 beats per minute, the number of respirations rose from 15 to 24, while the CO_2 in the chamber had gone up from 0.04 per cent. to 1.73 per cent. When the CO_2 reached 3 per cent. the circulation began to weaken, the heart action was slower and the respiration accelerated. From these experiments the investigator concluded that confinement in an air-tight room, even for a short time, had a clearly demonstrable influence upon man which was to be attributed to the carbon dioxide. When later he found that the respiration became difficult in an atmosphere indicating about 10 per cent. of CO_2, it was thought that these results should be attributed to other conditions rather than to an excess of carbon dioxide.

Friedländer and Herter,[20] in 1878 to 1879, found that the breathing of an atmosphere containing 20 per cent. CO_2 for an hour had no toxic effect; it produced, however, an irritation of the respiratory center and an increase in the heart action. Only when a gas mixture of 30 per cent. or more of CO_2 was breathed were signs of depression observed. In a study of the action of different amounts of oxygen they found[21] that there is an effect on the respiratory rhythm when the oxygen content is 15 per cent.; dyspnea in the experimental animal is only noticeable at 7 per cent. of oxygen and it succumbs only when the oxygen reaches from 2.1 per cent. to 3.8 per cent.

Seegen and Nowack,[63] in 1879, passed oxygen, free from carbon dioxide, into a chamber in which animals were caged; death resulted. Since the amount of carbon dioxide was not large, they could not explain the death of the animals as due to this gas, hence they assumed it was due to a poison from the breath. When this contaminated air was passed over ignited copper oxide, they found that the air lost its toxicity. From the result of this experiment they assumed that in addition to the known respiratory products, animals also expire a poisonous organic substance which becomes harmless when heated. The results observed by Seegen and Nowack were explained as due to impurities in the oxygen employed, in particular chlorine gas.

Remsen,[53] in 1880, performed some experiments to determine whether "albuminoid ammonia" can be regarded as a safe measure of the purity of air. Air was contaminated by being drawn through water containing decayed meat. The odor of this air was almost unbearable and according to all commonly accepted notions, it could not have been inspired without serious results; yet, when examined by the method used in all other cases, a very small amount of "albuminoid ammonia" was obtained, in fact, rather less than from ordinary air. These experiments were frequently repeated, and always with practically the same results. In other experiments[54] Remsen found that air contaminated by being drawn over comparatively dry, decaying organic matter, as well as air contaminated by respiration, yields more than the usual quantity of albuminoid ammonia. Attention was called to the fact that the simple statement that a given sample of air yields an abnormally large quantity of albuminoid ammonia, is not sufficient to enable us to draw conclusions with reference to the purity of air, but that the details regarding the source of the sample, season of the year, etc., must also be considered.

Hermans,[29] in 1883, repeated some of the work of R. A. Smith.[64] His experimental chamber, which was made of tin, was 1.2 meters in length, 1.6 meters in width and 1.8 meters in height. The subjects he selected were persons clean in body and clothing. The first ill effects occurred when the CO_2 rose above 3 per cent. Dyspnea occurred when the CO_2 content reached 5.3 per cent. After absorption solutions were used for CO_2, no hardship was felt on remaining inside the chamber, even when the oxygen was decreased to 10 per cent.

In other experiments attempts were made to discover the presence of organic matter in vitiated air. On passing air through standard sulphuric acid, the titer was always found to be unchanged. Three to four liters of air were passed over ignited copper oxide; the CO_2 and water content were no higher than in the unignited air. In the same way there was no CO_2 or water formed when the air, which had been freed from CO_2 and water, was passed over ignited copper oxide. The titer of a boiling acid or alkali solution remained unchanged even on long passage of many liters of contaminated air obtained toward the end of the experiment. The sodium hydroxide used for the absorption of the CO_2 was likewise evaporated and heated without any appearance of blackening or the formation of odorous vapors.

Hermans attributes the unpleasant sensation that some subjects had on entering the experimental chamber at the end of an experiment, to high temperature and to excess of moisture. He concludes that in healthy people no appreciable quantity of volatile combustible matter is given off with the expired air, and that if such contamination does occur it is due to gas formation in the intestine as a result of deficient digestion, or through the decompostion processes on the surface of the body due to uncleanliness. He mentions the necessity of ventilation for the removal of humidity and for the maintenance of a proper temperature.

Brown-Séquard and D'Arsonval,[10] in 1887, announced that the expired air of men and dogs contains a toxic substance. They pointed out that (1) the expired air almost always contains ammonia, perhaps under all conditions; (2) this air contains a very small quantity of organic matter which, if not already putrified on leaving the respiratory tract, has a great tendency to be rapidly altered even at normal temperatures; (3) the confined air laden with pulmonary exhalations is very harmful even if it contains only 1 per cent. of CO_2, with a corresponding decrease in the proportion of oxygen, while atmospheric air having the same proportion of CO_2 and a similar diminution in oxygen but not containing the pulmonary exhalation, is hardly a cause of any disturbance of the health. These facts, particularly the last, according to their reasoning, rendered it possible that expired air contains one or several toxic substances. To demonstrate this toxic substance they carried out three series of experiments on rabbits weighing between 1800–1900 grams. In the first series they injected into the vascular system of a rabbit, 4–6 cc. of a fluid obtained after the injection of 15–25 cc. of pure filtered water into the trachea of a dog or rabbit. In a second series they injected 6–7 cc. of the condensed expired breath of a man into an artery or vein of a rabbit. In the third series quantities ranging from 4–16 cc. of a liquid obtained by condensing the breath of a tracheotomized dog were injected, immediately after filtering, either into the jugular vein or the carotid artery of a rabbit.

The symptoms observed were: (1) dilation of the pupils, (2) slowing of the respiration, (3) a very rapid lowering of the temperature in the first few hours varying from 0.5° to 5° C. followed by a subsequent increase, (4) a paralytic weakness especially in the hind limbs, (5) increase of the heart action, the pulse frequently rising to 240, 280 or even 320 beats per minute, which lasted for several days or even weeks. The authors state that the effects could not be attributed to similar quantities of distilled water for, as Bouchard[9] states, distilled water does not begin to be toxic until more than 90 cc. per kilogram of the animal (rabbit) is injected.

When injections were made with larger quantities, 20 to 25 cc., of pulmonary liquid, the effects were more pronounced; the respiration became difficult, pupils were contracted and there was violent retching, sometimes there were general convulsions, often the animals had cramps, choleraic diarrhea came rapidly, often lasting until death, which usually took place in three or four days following the operation, at the most in four or five weeks. Post-mortem appearance showed a considerable congestion of all the viscera and especially the lungs. No embolism or infarct was noticeable.

These investigators believed they had discovered a strong poison of pulmonary origin in the exhaled breath and that it was probably this toxic agent that rendered confined air dangerous. They further believed that this poison was of the nature of an alkaloid or ptomaine.[11]

Jackson,[36] in 1887, in a preliminary communication announced the presence of CO in expired air, which he believed was present in quantities sufficient to explain the injurious influence exercised by expired air on animal life. Details regarding his procedure were not mentioned No other reference to the subject has been found.

In an additional communication,[12] in 1888, Brown-Séquard and D'Arsonval report that seven rabbits, weighing about two kilograms each, were injected subcutaneously with 20 to 40 cc. of condensed fluid from the lungs of two dogs. The result was the same as in the previous experiment. Five rabbits died very quickly, four within twenty-four hours and one after thirty-six hours. Two of the rabbits that survived were very weak and it appeared that they would not live long. The phenomena were the same as in previous experiments.

To show that it was a chemical (organic) and not a bacterial poison they injected into the blood stream or subcutaneously some of the pulmonary condensation from a dog. This fluid was boiled in a closed vessel before the injection. The results were the same as those with the unboiled liquid. It seemed to Brown-Séquard and D'Arsonval that the boiled liquid was even more toxic than the unboiled liquid. They concluded that the substance responsible for the toxic action is an alkaloid, for the following reasons: (1) The condensed fluid was alkaline. (They first described it as neutral.) (2) The condensed fluid does not lose its toxicity after boiling in a closed vessel. (3) On subcutaneous, intravenous or intraärterial injection into rabbits symptoms appear which are similar to those which result from the injection of alkaloids. The organic nature of the pulmonary poison was established from the fact that the pulmonary condensation gives a yellow color with pure concentrated sulphuric acid, and that it reduces ammoniacal nitrate of silver as well as chloride of gold.

This work of Brown-Séquard and D'Arsonval was followed up by Wurtz[74] in 1888, who undertook to throw some light on the chemical nature of this toxic substance. To recover and isolate the volatile bases which were believed to be eliminated by the respiratory tract, expired air was passed through a 1 per cent. solution of oxalic acid contained in a special apparatus in which the errors due to the projection of saliva or solid particles are eliminated. After the air had passed through for a sufficiently long time, the excess of oxalic acid was neutralized with freshly precipitated, pure calcium chloride. The filtrate was exactly neutralized with weak hydrochloric acid and evaporated in a vacuum. The residue yielded ammonium chloride which predominated, and the hydrochloride of an organic base. This latter substance had the following properties: It is precipitated by Bouchardet's reagent and potassium mercuric iodide; it forms a soluble chloroplatinate, crystallizing in short needles, and a soluble salt with auric chloride. The solution of the hydrochloride of this base warmed at 100° C. gave off a specific aromatic odor. For want of a sufficient amount of the substance Wurtz was unable to make any analysis or to test its physiological action.

There was an immediate attempt made to obtain results which corroborate those of Brown-Séquard and D'Arsonval. Dastre and Loye,[16] in 1888, exposed one dog to the expired breath of another dog for about $6^1/_2$ hours without any result. When the expired breath of a tracheotomized dog was passed through cooled glass spirals and the condensed liquid thus obtained was injected intravenously or subcutaneously into rabbits, dogs or frogs, no symptoms of poisoning were observed in the majority of cases. They observed one death during the injection, into a rabbit, of 190 cc. (60 cc. per kilo) of the pulmonary exhalations of a dog, yet, by control experiments with pure sterilized distilled water they obtained a more remarkable result—a rapid death from the injection of distilled water when the amount injected was only 25 cc. per kilogram. They found that 50 to 70 cc. of the condensed fluid of respiration (20 to 35 cc. per kilogram) could be injected into the veins of the ear of a dog without any of the symptoms reported by Brown-Séquard and his collaborator.

Hoffmann Wellenhoff,[33] in 1888, repeated some of Brown-Séquard and D'Arsonval's experiments. For condensation of the breath he used a two-liter flask packed in ice and connected this with a similar inverted flask in which there was a spiral glass tube that ended just below the stopper of the first flask. This spiral was also packed in ice. With this arrangement any moisture that escaped condensation in the first flask was condensed in the spiral and dropped into a flask below. In order to prevent the condensation of water before entrance into the receiver and to keep out bacteria or saliva, the air was first passed through a U tube warmed to 37° C. and provided at one end with a cotton plug. This tube was connected with a rubber tube into which the subject breathed. The entire apparatus was sterilized before the experiment by heating at 140° C. The condensed fluid was neutral in reaction.

When large quantities of this fluid (28 cc. into a 1200 gram rabbit) were injected intravenously at 12° instead of at 37° C. results were obtained which resembled those of Brown-Séquard and D'Arsonval, but the animals remained alive. Under such conditions Hoffmann-Wellenhoff observed muscle-weakness, slowing of respiration, fall of body temperature and dilation of the pupils. These symptoms disappeared at the end of twelve hours.

On injecting the rabbits with 6 to 30 cc. of the fluid at 37° C. all the results were negative. Three animals were injected in the jugular vein, one receiving 28 cc. of the condensed fluid, another 25 cc. of distilled water, and a third 50 cc. of distilled water. There was no difference in the symptoms of the animals. As a result of his work Hoffmann-Wellenhoff concluded that under normal conditions of health there is no volatile poison in the breath of man.

Russo-Giliberti and Alessi,[59] in 1888, carried out a modification of Brown-Séquard and D'Arsonval's experiment. Their experiments have a special interest because they were made under conditions which most nearly approach those of daily life. Instead of directly condensing the expired breath on its exit from individuals, they passed the expired air from a school-room during the period of instruction over a large cooling apparatus. The openings of the room were carefully closed; thus a vitiated atmosphere was obtained which, after two hours, gave a sense of oppression to a person coming in from the open air, and respiratory disturbance and headache to the occupants. The liquid collected was filtered and kept till the next day, care being exercised to maintain the liquid in an aseptic condition. That these efforts were successful was shown by the fact that the liquid showed no growth in a suitable medium. Guinea pigs were injected with amounts varying from 1.9 to 78.2 grams per kilogram. No toxic effects were observed. These results, as well as those of Dastre and Loye, are entirely opposed to those of Brown-Séquard and D'Arsonval. Russo-Giliberti and Alessi were inclined to believe that Brown-Séquard's results were due not to intoxication but to infection by germs.

Bechamp,[3] in 1888, announced that he found in the condensed liquid from expired air a fixed product capable of playing the rôle of a ferment, and that this substance can convert starch into soluble starch. He estimated that in 100 grams of condensed expired air dried at 60° C., there was 0.01 gram of fixed organic matter. He stated that previous investigators had shown some enzymes to be toxic when injected intravenously, and from this he concludes that the zymase of the expired air may help explain the toxicity affirmed by Brown-Séquard and D'Arsonval.

Uffelmann,[66] in 1888, reported the results of a study of the oxidizable matter in the air. It was found that the air of a bed-room in which a grown person had slept the whole night, consumed more potassium permanganate than the air of the previous evening, before the occupation of the room. The air of an auditorium before an address was found to require less potassium permanganate for oxidation than after the address. Uffelmann proposed that the quantity of potassium permanganate required for oxidation might be used as a measure of the vitiation of the air.

Brown-Séquard and D'Arsonval returned no direct answer to Dastre and Loye, Hoffman-Wellenhoff or Russo-Giliberti and Alessi, who denied the existence of a volatile poison in the breath, but a little later, in 1889,[13] they reported a new ventilation experiment supporting their previous claim.

Rabbits were confined in a series of metallic cages connected in series by means of rubber tubing, and through which a constant stream of air was drawn. The animal in the first cage received pure air, while all the other animals breathed air that was more and more contaminated by the products of respiration from the previous cages. The cages consisted of vertical cylinders of galvanized tin with a false bottom of latticed wire, and were large enough to give ample room for a large rabbit. The bottom of the cylinder ended in a funnel-shaped cone which was connected with a vessel for the reception of the excreta from the animal, such that this matter would not stagnate in the cage. Of eight young rabbits, five to seven weeks old, placed in such a series of cages, all died except the first two. In general, the animal in the last cage with the greatest impurity died first, and so on, in the order of the impurity of the air of the cages. Some rabbits in the last two cages resisted death for from four to six days. Usually death took place in a week in the fourth cage and scarcely a few days more in the third. The rabbits in the first two cages survived for a long time and died only as the result of accident.

Brown-Séquard and D'Arsonval could not attribute the death of these animals to an excess of CO_2 in the atmosphere of the cages, for they rarely found more than 2 to 3 per cent. of this gas in the last jar with small animals or 4 to 6 per cent. with larger animals. To demonstrate that it was not the CO_2 that contributed to the death of the animals, they ascertained that pure CO_2 could be inhaled in fairly large proportions in atmospheric air by man, dog, rabbit and other mammals. The investigators themselves were able to breathe, for more than one or two hours, air containing over 20 per cent. CO_2 without being inconvenienced in any marked manner. They also state that the dogs were able to breathe, without dying, a gaseous mixture containing 95 per cent. CO_2 and 5 per cent. of atmospheric air for more than 12 minutes.

That the death of the animal was due to a volatile pulmonary poison, and that they had a new proof for the innocuity of carbon dioxide, was shown by placing absorption tubes containing concentrated sulphuric acid between the last two cages; the animal in the last cage remained alive while that in the cage before it was the first to die. As many who denied the existence of a pulmonary poison, raised the objection that it was possibly the evaporations from the excreta that was a cause of death, Brown-Séquard and D'Arsonval[13] carried out an experiment in which they exposed a rabbit in the last of a series of six cages, not only to the pulmonary exhalations from the animals in the preceding cages, but to the exhalations of their excreta as well. The rabbit showed no effect even after three months.

Geyer,[24] in 1889, conducted experiments on the toxicity of expired air. He condensed the expired air of a healthy person by passing it through cooling tubes. The liquid thus obtained was injected subcutaneously into a rabbit. No toxic symptoms appeared. Lipari and Crisafulli,[40] in 1889–90, likewise reported results that were directly opposed to those of Brown-Séquard and D'Arsonval. They could find no organic principle possessing toxic properties in the expired breath of healthy persons.

Lehmann and Jessen,[39] in 1890, repeated some of Brown-Séquard and D'Arsonval's injection experiments and studied the chemical properties of the condensed fluid and the effects of confined air on man under certain, special conditions. For the collection of the condensed fluid they used a glass spiral packed in ice. Before the glass spiral they placed an empty wash bottle to keep back any possible saliva. Immediately before the glass spiral a Woulf bottle was placed in a water bath to avoid premature condensation of water. 15–20 cc. of condensed fluid were collected per hour.

The fluid was always clear as water, odorless and neutral in reaction. Nessler's reagent showed the constant presence of ammonia. In persons with good teeth, the amount of ammonia was small, while in case of bad teeth, the ammonia was larger in amount but never more than 10 mg. of ammonium chloride per liter. Traces of hydrochloric acid were also constantly present. On evaporating the filtered condensed fluid in glass bottles a small residue always remained, ranging from 39 to 86.4 mg. per liter of fluid. The residue consisted of characteristic microscopic crystals, belonging to the rhombic system; plates and columns prevailed. These crystals were insoluble in alcohol and ether, difficultly soluble in water, easily soluble in dilute hydrochloric acid and on treatment with sulphuric acid were slowly converted, without effervescing, into needles of calcium sulphate. On allowing 52.6 cc. of distilled water to stand in a glass spiral over night they found on evaporation a residue of one milligram of crystal (about 20 mg. per liter) quite similar in form and properties to the one just described. The crystals, therefore, did not originate from the condensed expired breath, but from the glass of the spiral, and were probably crystals of calcium silicate. In a number of experiments Lehmann and Jessen tested the reducing power of the organic matter in the breath by titrating it with dilute potassium permanganate in presence of sulphuric acid. In two control tests on the condensed fluid of the same experiment, one showed 3.6 mg. of oxygen for the oxidation of one liter; the second, 4.2 mg. of oxygen. The first cc. of distillate of 40 cc. of condensed fluid consumed 0.06 mg. of oxygen.

The presence of an alkaloid in the condensed fluid or in its distillates could not be shown. The following reagents were used in the tests: Platinic chloride, auric chloride, potassium cadmium iodide, potassium iodide, Bouchardet's reagent, potassium dichromate, picric acid, metatungstic acid and phosphotungstic acid. Mercuric chloride produced an opalescence several times, the coloration of which, like the yellow coloration with Nessler's reagent, indicated traces of ammonia. Following Wurtz's directions they were unable to get a filtrate free from lime and oxalic acid. The evaporation of the hydrochloride gave them only calcium chloride and ammonium chloride. These investigators also failed to get any ammoniacal silver reduction as stated by Brown-Séquard and D'Arsonval.

Injections of the condensed fluid into animals gave negative results, as did the injections of from 16–40 cc. of fluid from the washings of the lungs. Their results were thus contrary to those of Brown-Séquard and D'Arsonval.

Hermans had shown that excretory products from the lungs and skin were not injurious, if the latter is clean and the person wears clean clothing. Lehmann and Jessen now took up the question of the effect of evaporations from the skin and clothes of a laborer who was dirty and perspiring as a result of his work. For this purpose they confined such a man in an air-tight zinc chamber having a capacity of 635 liters. Only his nose and mouth were connected with the outside air. After keeping this man in the chamber for a half hour at 24° C. and his clothing were soaking with perspiration, they confined a girl of 12 years on one occasion and a boy of the same age on another occasion, in the atmosphere for two hours. In one case there was a complaint about the bad odor of the air, but after ten minutes this odor was no longer perceptible. The respirations gradually rose from 18 to 30 per minute. There was no trace of headache or other disorder. In the other case the respirations rose quickly to 30, then to 42 per minute. There were no ill effects.

Lehmann and Jessen explained the discomfort of badly ventilated concert halls as due to high temperature, high humidity, lack of motion of the air, and odors; and if in addition to these a person has a weak constitution or idiosyncratic sensitiveness, then fainting, etc., is easily explained. They pointed out that the physical factors, temperature, humidity and air motion, play just as important a part as the composition of the air.

Margouty,[42] in 1891, reported the results of experiments similar to those of Hammond and also of experiments in which the fluid from the expired air was injected into animals. His results did not correspond to those of Hammond and there was no evidence for the toxicity of the injected fluid.

Haldane and Smith,[25] in 1893, studied the physiological effects of air vitiated by normal respiration. They used a wooden air chamber, 6 feet 2 inches high, 2 feet 11 inches wide, 3 feet 11 inches long, having on one side a large window for the admission of light and for purposes of observation. The samples of air for analysis were drawn through a tube placed in the wall of the chamber about three feet from the floor. In one experiment which lasted about eight hours the CO_2 rose from 0.03 to 6.39 per cent.; the oxygen fell from 20.9 to 13 per cent.; the temperature of the room rose from 57.9° to 66° F. and the respirations from 18 to 30. On leaving the chamber the subject of the experiment had a violent headache. He was well the next day. An observer from outside, on entering the chamber as it was vacated by the subject, felt no perceptible discomfort or nausea, but the respirations rose after a few minutes to 25, and were very deep; the pulse was 92. It is interesting to note that air vitiated to such an extent as to completely prevent a match from burning had no appreciable effect upon the subject of the experiment. For the purpose of excluding the effects of the increased proportion of carbonic acid, the experiment was repeated, using soda-lime to absorb the carbon dioxide. The effects noted could, therefore, be ascribed to the diminution of oxygen along with the presence of organic matter. In this experiment which lasted twelve hours the CO_2 increased from 0.0 to 1.4 per cent.; the oxygen went down from 20.9 to 13 per per cent.; the pulse from 90 to 84; the body temperature from 99° to 96.9°F.; the chamber temperature rose from 53° to 71° F. and the respirations from 17 to 18. No headache was noticed either during the experiment or on leaving the chamber, nor was there any sense of relief in breathing fresh air again. An observer who entered the chamber at the end of the experiment could perceive no smell of organic matter, nor any tendency to hyperpnea. The air was still so highly vitiated that it was quite impossible to light a match.

Haldane and Smith repeated some of the injection experiments of Brown-Séquard and D'Arsonval. Before doing these experiments they performed some comparative experiments with boiled distilled water, and found that with rabbits weighing 1800 grams, hematuria was apt to occur when the amount injected passed much beyond 100 cc. They, therefore, took 80 cc. as their maximum dose. To obtain the condensed liquid from the lungs, they expired through a Liebig condenser, in the jacket of which there passed a constant stream of cold water. The condensation liquid was collected in a flask, the bulb of which was buried in ice and when the required amount (80 cc.) had been obtained, it was at once injected into the subcutaneous tissue of the back of a rabbit. Six rabbits were injected with 80 cc. each but no disturbance in the health in any one of them was observed, with the exception that in one case, there was a slight hematuria. They, therefore, concluded that the physiological effects due to the injection of the condensed liquid do not appreciably differ from those produced by distilled water. Further, they concluded that (1) the immediate danger from breathing air highly vitiated by respiration arises entirely from the excess of carbonic acid and deficiency of oxygen, and not from any special reason; (2) the hyperpnea is due to excess of carbonic acid and is not appreciably affected by the corresponding deficiency of oxygen. The hyperpnea begins to appear when the carbonic acid rises from 3 to 4 per cent., and at about 10 per cent. there is extreme distress; (3) hyperpnea from deficiency of oxygen begins to be appreciable when the oxygen in the air breathed has fallen to a point which seems to differ in different individuals.

Brown-Séquard and D'Arsonval's second challenge to the scientific world issued by the publication of their ventilation experiment was soon taken up by a number of investigators. Merkel,[43] in 1892, repeated some of Brown-Séquard and D'Arsonval's experiments. He was unable to confirm their injection results but supported their ventilation experiments. Condensed fluid from the breath was collected by first allowing the expired air to pass through a Woulf bottle standing in water at 37° C. A wide curved tube, 1.2 meters long, packed in ice, led downward from the

Woulf bottle and terminated as a straight tube pointing downward and passing through the stopper of a receiving bottle. At the point where the wide curved tube turns straight downward, it was connected with a vertical glass tube of similar bore also packed in the same ice receptacle as the other, and was connected at its upper end with a gas meter. The amount of fluid collected was 15 cc. per hour per person. The apparatus was sterilized before use. During the course of a day 2500 liters of air were breathed in and 100 cc. of condensed fluid collected.

Tests on the fluid showed ammonia to be present. Silver nitrate caused no change. On injection of 3 cc. of the fluid into a mouse, or 70 cc. into a small rabbit the animals were not affected. On evaporating the condensed fluid from the passage of 2400 liters of expired air down to 5 cc. in a vacuum at 39° C. ammonia was still present. Alkaloid tests were negative. Silver nitrate was not reduced. These results agreed with those of Lehmann and Jessen,[39] and were opposed to those of Brown-Séquard, and D'Arsonval[11] and Wurtz[74].

For his ventilation experiment, Merkel used white mice which were confined singly in 4 air-tight glass vessels of $1^1/_2$ liters capacity. These vessels were connected by means of glass tubes. Between the third and fourth vessels a Geissler absorption tube containing sulphuric acid was interposed. Air was now drawn through slowly by means of an aspirator such that the second mouse breathed the air from the first cage, the third from that of the second cage, etc. The result was, just as in Brown-Séquard's experiment, that the mouse in the third cage died first, after 16–20 hours, while that in the last cage remained alive.

The symptoms presented by the mice which succumbed were: First, restlessness, gradual accelerated respiration, then slowing of respiration and finally, spasmodic deep respirations becoming constantly less frequent until, finally, death occurred. He could not observe any difference in these symptoms and those of CO_2 poisoning, but he states the per cent. of CO_2 in the cage, as being a maximum of 1.5 per cent.

Merkel also conducted expired breath through 0.1 per cent. HCl, with the idea of combining the organic matter with it, and believed he was successful, but the quantities of the salts produced were so small that a determination of their chemical nature was impossible. His experiments upon animals with this body, obtained from its combination by neutralizing the acid, gave negative results. No toxic effects were obtained on treating mice with the residue from the evaporation of the condensed fluid, when the same was taken up in a small quantity of water. The distillate obtained on treating the residue from the evaporation with an excess of alkali also had no effect on mice. Merkel concludes that the expired breath of healthy persons contains a volatile poison in extremely small quantities, being probably a base which is poisonous in the gaseous state but loses its toxicity after combination with acid.

Haldane and Smith,[26] in 1893, repeated Brown-Séquard and D'Arsonval's ventilation experiment. Five bottles, each having a capacity of 1$^1/_2$ liters, were connected in a series. A mouse was placed in each bottle and ventilation established through the whole system by means of a filter pump, a small gas meter being placed between the last bottle and the pump. Specimens of the air leaving the last bottle were drawn off at intervals for the purpose of the analysis. Full grown mice were used. The mice originally placed in the last two bottles had been exposed to the full effects of the vitiated air for 53 hours. The ventilation was from 12–26 liters per hour. 5.2 per cent. CO_2 in the last bottle corresponded to a ventilation of 12 liters per hour in the early part of the experiment while a CO_2 content of 2.8 per cent. corresponded to a ventilation of 26 liters. The experiment lasted three days. All the mice were normal.

In a second experiment, lasting 30 hours, an absorption tube containing pumice stone soaked in sulphuric acid was placed between the fourth and fifth bottles. The ventilation in liters per hour was from 12 liters to 8$^1/_2$ liters with corresponding amounts of CO_2 from 6.6 per cent. to 5.4 per cent. No serious effects were observed. They now reduced the ventilation still further, retaining the absorption tube between the fourth and fifth bottles. At a ventilation of 8 liters per hour, the CO_2 in the last bottle was 6.6 per cent. Signs of discomfort were observed after about five hours when the ventilation was $5^1/_2$ liters and the CO_2 in the last bottle was 10.2 per cent. The fourth and fifth animals seemed to be panting, the fifth being less lively than usual. Haldane and Smith state that these experiments, like their former ones on rabbit and man, are distinctly against the theory that a volatile poison, other than carbonic acid, exists in expired air. They explain the survival of the animal in Merkel's last cage as probably due to a leakage in the connections of his last vessel or of his absorption tube.

Beu,[7] in 1893, collected expired air with an apparatus very much like Merkel's. He took the precaution to clean his apparatus with a solution of potassium permanganate and distilled water and then sterilized it before use. The amount of air expired measured by a gas meter was found to be 3000 liters in eight hours, from which he collected 100 cc. of fluid. The latter was clear and on removal of the stopper gave out a peculiar but not unpleasant odor, which, after a few days, became rather fungoid. Ammonia was found to be present. Silver nitrate failed to show the presence of of chlorine. Its reducing power upon a solution of potassium permanganate showed that 50 mg. of oxygen were necessary to oxidize one liter of fluid, or 15 mg. in twenty-four hours, which denotes 0.0017 mg. per liter of expired air. Alkaloid reactions were negative.

In another experiment 500 liters were expired through 150 cc. of a 1 per cent. solution of HCl. On evaporating this liquid to dryness on the water bath, a yellowish brown residue remained, whereas, on similar evaporation of a control solution with an equal volume of acid there was practically no residue. The residue obtained above was dissolved in distilled water and the entire quantity (1.5 cc.) was injected subcutaneously into the back of a white mouse without producing any noticeable effect. On passing 700 liters of air through 150 cc. of a 0.5 per cent. solution of HCl, evaporating the solution on the water bath, dissolving the residue in water and testing for alkaloids, a negative result was obtained. From his experiments Beu concludes that organic substances are present in the expired air, but in such small quantities that it must be assumed that these substances are less likely to vitiate the air of a confined space than the other exhalations from the body surface, such as fatty acids or intestinal gases, etc.

Beu next confined a mouse in an air-tight glass vessel for three hours while he breathed into the vessel, 3200 expirations. To keep CO_2 from the vessel there was attached, before the entrance to the same, a tube containing pumice stone with potash solution. The animals showed no noticeable effects. In a second experiment lasting four hours, the CO_2 was not absorbed. Here, too, the animals were not affected.

The Brown-Séquard ventilation experiment was repeated, using four connected cages, each containing a white mouse. After twelve hours, increase in frequency of respiration was noticed. After twenty hours all were dead. No mention is made of the circumstances or of the order in which the animals died. Beu believed that death occurred on account of the low temperature of the unheated room (8° C.), together with the increase in humidity in the air of the cages, for moisture was condensed on the inner walls of the glass cages. He also believed that the protection offered by the sulphuric acid in Brown-Séquard and D'Arsonval's experiment was due to its abstraction of the moisture of the air.

Rauer,[51] in 1893, repeated the Brown-Séquard and D'Arsonval ventilation experiment and also made some modifications of it. He confined white mice singly in each of four connected glass vessels of about $1^1/_2$ liters capacity. Through the tight paraffined cork, three tubes passed, one reaching down near the bottom served for the entrance of the air; the second terminated just below the cork and served for the exit of the air; the third, which reached down as far as the animals, was usually closed and served for the removal of air for chemical analysis.

In a preliminary experiment, in which the ventilation amounted to 11–12 liters per hour, the animals remained alive eight days or more without any noticeable symptoms. In another experiment in which five animals were used, the ventilation was reduced to four liters, but symptoms did nor appear until after some days. At first there was a moderate acceleration of respiration, which was followed by a slowing of respiration and later by spasmodic breathing. The animals which were at first restless, later sat quietly and whenever they made any voluntary motions, these were slow and without energy. There was a tendency to fall on the side until, finally, the animal remained on its side, motionless, with the extremities stretched full length, and died in that position. These symptoms could not be distinguished from those of CO_2 poisoning.

Whereas Brown-Séquard and D'Arsonval announced that the CO_2 content in the last cage seldom rose to over 3 per cent. and Merkel found that the CO_2 in the last cage amounted to 2 per cent. in the highest case, Rauer states that after five hours the CO_2 content of the fifth cage amounted to 9.3 per cent. and on further reducing the speed of ventilation it even rose to over 14 per cent. Rauer's high results here correspond to those of Haldane and Smith.

In another experiment with six animals and with a ventilation of $2^1/_2$ liters per hour, Rauer inserted four absorption tubes containing soda-lime between the last two vessels and a Geissler tube containing sulphuric acid between the fourth and fifth vessel. The sixth animal remained alive while the fifth died earlier than the fifth animal in the first experiment. However, when the soda-lime was allowed to become saturated with CO_2 so that it could not absorb any more, it no longer saved the last animal from dying. The insertion of the vessel with sulphuric acid, which, according to Brown-Séquard and D'Arsonval as well as according to Merkel, had a protecting effect by holding back poisonous respiration products, had no effect when used by Rauer.

Symptoms of poisoning in animals occurred when the CO_2 content reached 12–14 per cent. Exactly the same symptoms were shown by an animal exposed to a mixture of pure air and 12–14 per cent. CO_2. The somewhat greater noxiousness of expired air as compared with room air mixed with pure CO_2, is explained by the diminished oxygen content of the former. Rauer concluded that there is no organic poison in expired air, death being due to the excess of carbonic acid in the cages.

Sanfelice,[62] in 1893, repeated a Hammond experiment. He confined two mice in a 5-liter flask through the stopper of which two tubes passed, one reaching down almost to the bottom, and the other ending just below the cork. The external parts of these tubes were provided with stopcocks. The CO_2 in the flask was absorbed by a solution of barium hydroxide. The apparatus and contents were carried to an inhabited, poorly ventilated shanty. 20–40 liters of the air of one of the rooms were passed through the shorter tube by means of an aspirator. The stopcocks were then closed and the apparatus taken at once to the laboratory, where a manometer was connected with the shorter tube and sufficient oxygen was passed into the other tube to cause the mercury of the manometer to descend one millimeter. The mice were dead in six or seven hours. A control experiment, which consisted in passing into the flask pure air under the same conditions as before, resulted in the same way, the animals dying in six or seven hours. From these results Sanfelice was unable to decide whether there was a toxic principle in vitiated air, but he thought that the death of the animals might have been due to excess of heat, due to lack of radiation. From an examination of air taken from various sources, Sanfelice finds no relation existing between the content in CO_2, organic matter, microörganisms and the degree of temperature of an enclosed space.

Lübbert and Peters,[41] in 1894, repeated the "Brown-Séquard experiments." A guinea-pig was placed in each of a series of four cages. A vessel containing soda-lime placed before the last cage saved the animals from CO_2 poisoning. Since the soda-lime might possibly keep back some acid poison, Lübbert and Peters substituted wash bottles of potassium permangate containing 1 per cent. sulphuric acid. This permanganate solution held back CO_2 just as effectively as soda-lime.

In another experiment the following arrangement was used: Between the third and fourth cages they placed a combustion tube, 75 cm. in length, through which the air from the third cage was conducted, passing over red hot copper oxide, to remove the organic matter. The heated air was cooled and the water condensed by passing it into a tall cylinder surrounded with ice. The water-free air was now passed through a series of twelve U tubes, 2 mm. internal diameter, having arms 80 cm. in length, forming altogether a cooling tube 20 meters in length, so that during the seventy-two hours of the experiment, the air entered the fourth cage at a constant temperature of 18° C.

Determinations of the CO_2 of the air from the third cage gave values of 10.8 to 11.1 per cent., and almost the same values (10.73–11.3 per cent.) were obtained from the air emerging from the long, cooling tube. The cage with the fourth guinea-pig contained from 11.2 to 12.9 per cent. CO_2. This animal died after 24 hours, the third also died but a little later.

That it was not the combustion arrangement that caused death is seen from the fact that a different animal placed in the fourth cage, using the same arrangement, remained alive, when, after the cooling tube, a soda-lime tube was inserted together with a control flask containing clear baryta water. This latter solution remained clear. With this arrangement the third animal died in 48 hours. The fourth animal was then receiving a CO_2 free air which contained only 11.4 to 12.6 per cent. oxygen, while the outgoing air contained 1.4 to 2 per cent. CO_2 and 10–10.2 per cent. oxygen. The results obtained by this arrangement substantiated the conclusion they had formed after conducting the experiment in the usual manner; that the cause of death was traceable to the high per cent. of carbonic acid. As the removal of the organic matter failed to save the life of the animal in the last cage, they decided that it had been conclusively established that there is no organic poison in the expired air.

Brown-Séquard and D'Arsonval,[14] in 1894, reported further experiments, giving fuller details as to their experiments and the apparatus employed. One experiment was carried out to show that the toxicity of the air was not due to exhalations from the excreta. A rabbit was confined for three months in a cage into which there passed the end of a long pipe, which was connected by side tubes to each of the excreta receptacles placed below the individual cages of a regular Brown-Séquard series. The emanations from all these dejecta were aspirated through the cage. The rabbit was not affected.

The authors stated that the pulmonary poison is more fatal to certain animals than to others, the differences in this respect being so great that some animals succumb at the end of a very short time (less than a day), while others die only at the end of a month or two, or may even show only an impairment of their health. As to the toxicity of CO_2, they state that it contributes in no way to death in their cages; in refutation of the danger of a considerable proportion of this gas, rabbits were caused to breathe carbon dioxide in amounts of from 6–8 per cent. for entire days without any bad effect. They still believed in the truth of their previous statements and could not understand the failures on the part of the other experimenters. Haldane and Smith's ventilation experiment was criticized on the ground that the duration of their experiment was too short.

Billings, Mitchell and Bergey,[8] in 1895, investigated the properties of expired air and its effects on animal life. They found no microörganisms, epithelial scales, etc., in the condensed moisture from air expired by men in ordinary quiet respiration. Tests for alkaloids were negative. Determinations were made of the ammonia and the reducing power upon a solution of potassium permanganate. In each case the amount of ammonia was very small. The following data were taken from the above report:

	Free ammonia	Albuminoid ammonia
Healthy man . . .	0.019	0.081
Man with tracheal fistula .	0.00046	0.00036
Consumptive . . .	0.003	0.0034

The average results on the reducing power, stated in milligrams of oxygen consumed per liter of condensed fluid, are as follows: Healthy man, 10.72; man with tracheal fistula, 13.49; consumptive, 19.34; average for five specimens of fluid condensed from the expired air of a healthy man four hours after he had taken a meal was 11.98, while the average for six specimens from the breath of the same man half an hour after a meal, was only 3.86; two specimens collected from the same man three and a half hours after a meal, but just after the mouth had been rinsed with thoroughly warm water, the average was 2.49. These results indicate that the ammonia and oxidizable organic matter in the condensed fluid were, to a large extent, due to products of decomposition of organic matters in the mouth.

The results of tests for the presence of an organic alkaloid in the condensed breath were negative and correspond to those reported by Lehmann and Jessen[39] and by Beu.[7]

Some experiments were made on the amount of oxidizable matters in atmospheric air. These results differed greatly, some showed a mere trace of organic matter, others yielded an amount which consumed from 0.204 to 0.558 gram of oxygen per 1000 cbm. of air. These differences were mainly attributed to the quantity of organic dust in the air in different places or in the same place at different times.

To determine the nature of the gaseous mixture in which small animals die with symptoms of asphyxia, Hammond's experiments were repeated. It was found impossible by this method to absorb all the carbon dioxide produced by an animal. In an average of eight experiments, the CO_2 had increased to 13.24 per cent., while the oxygen had diminished to 4.67 per cent. The symptoms observed were those produced by insufficiency of oxygen, and there was no evidence that death was due to organic matters in the air. The duration of life in the animals confined was from three to six hours, being much longer than that reported by Hammond (less than an hour), who used a slightly smaller vessel. Brown-Séquard and D'Arsonval claimed that the method of absorbing carbonic acid by an alkali changes the organic poison which they claim to be present, hence, these experiments are not conclusive on this point.

Another series of experiments was made upon mice and sparrows to determine the time required to produce death by asphyxia when the animal is confined in a jar of known capacity, and no provision is made for removing carbonic acid and moisture, or for supplying fresh air; determinations were also made of the proportions of carbonic acid and of oxygen existing in the enclosed air at the time of death. As a general rule the animal dies when the carbonic acid has increased to between 12 and 13 per cent. and the oxygen has diminished to between 5 and 6 per cent.

Experiments were carried out on different animals (mice, rabbits, guinea-pigs and sparrows) to determine whether death is due to the increase in the carbonic acid, or to the diminution of oxygen or both. They found that the most important factor in producing death was the diminution in oxygen in the inspired air, and that so long as the oxygen is present in the proportion of 6 per cent. and upwards, carbonic acid may be present to the amount of 20 per cent. without causing death. When much more than 20 per cent. of the mixture is in the form of carbonic acid, say 30 to 40 per cent., at least 12 per cent. of oxygen must be present to preserve life. When the proportion of oxygen in an artificial mixture was reduced, the duration of life in the experimental animal was shortened. Mice weighing between 15–18 grams were placed in jars of 2280 cc. capacity, and exposed to a mixture of gases consisting of 0 per cent. CO_2, 11.35 per cent. oxygen and 88.65 per cent. nitrogen. The animals died in from $3^1/_2$ to $4^1/_6$ hours. The air then contained between 6.56–7.52 per cent. CO_2 with amounts of oxygen corresponding to 4.14 per cent. to 3.16 per cent. These results correspond with those obtained with a mixture of gases by Paul Bert[6], who concluded that carbonic acid when inhaled is really a poison, and with the results of Friedländer and Herter[20] to the same effect.

The results of experiments on mice made with air and with different mixtures of gases, at different temperatures, show that the duration of life in confined places is influenced to a very marked degree by temperature, and that this influence is independent of the richness of the air in oxygen. For example, an atmosphere consisting of 90 per cent. of oxygen and 10 per cent. of nitrogen, does not support life quite as long as does ordinary atmospheric air when the temperature is 0° C., while at a temperature of 50° C. the atmosphere rich in oxygen supports life much longer than the ordinary air.

In another series of experiments animals were injected with the fluid condensed from the air expired by healthy persons and by a man with a tracheal fistula, from whom it was possible to obtain such fluid without contamination from the exhalations of the mouth. The fluid was collected in sterile apparatus and subsequent cultures made from it indicated that it was sterile. Before injection it was warmed to about 35° C. The injections were made into the general circulation in rabbits, and into the peritoneal cavities of rabbits, guinea-pigs and white rats, using the technic of Brown-Séquard and D'Arsonval[12] and of Hoffman-Wellenhoff.[33] The results agreed with those of the investigator last mentioned, indicating that the fluid condensed from pulmonary exhalations of man has no toxic or specially injurious effect when injected into animals, and that there is no evidence that such fluid contains an organic poison. A number of repetitions of the Brown-Séquard and D'Arsonval ventilation experiments were made on sparrows, mice, guinea-pigs and rabbits. Attention was called to the difficulty of keeping the apparatus absolutely air-tight and to the fact that some of the discrepancies in the results, at least for the earlier experiments, were due to slight leakage of air through some one or more of the numerous joints. Haldane and Smith[26] had already noted this difficulty. The more important results in the later experiments indicate that these defects had been remedied.

In the great majority of cases reported by Billings, Mitchell and Bergey, death was evidently due to the diminution in the oxygen and increase in the carbonic acid. The oxygen was reduced to between 4 and 6 per cent. and the carbonic acid increased to from 12 to 14 per cent. The insertion of absorption tubes containing caustic alkalies between the bell-jars to absorb the carbonic acid, and of concentrated sulphuric acid did not give results corresponding to those reported by Brown-Séquard and D'Arsonval.

It was shown by Bernard[5] that if animals became somewhat habituated to the conditions under which they were placed, they could live in an atmosphere which would be fatal to a fresh animal. Billings, Mitchell and Bergey also showed this by alternately keeping and removing a number of mice in a series of Brown-Séquard cages for a period of about two to four days, and now and then inserting a fresh mouse in one of the occupied cages, usually toward the end of the series of cages. The fresh mouse would usually die in a few minutes. The duration of the entire experiment was about six weeks. In the case of several mice, this power to resist the foul atmosphere was preserved for from three to eight days after they had been removed from the jar. They had acquired a certain degree of permanent immunity. The results indicate the possibility of developing immunity to foul atmospheres.

Attention is also called by Billings, Mitchell and Bergey to the great difference in the individual susceptibility of different animals. Among their conclusions are the following: "The proportion of increase of carbonic acid and of diminution of oxygen which has been found to exist in badly ventilated churches, schools, theatres or barracks, is not sufficiently great to satisfactorily account for the great discomfort which such conditions produce in many persons, and there is no evidence to show that such an amount of change in the normal proportion of the gases has any influence upon the increase of disease and death rates, which statistical evidence has shown to exist among persons living in crowded and unventilated rooms." They admit that such an atmosphere may affect the vital resistance of persons to ward off disease, and call attention to the necessity for research on that point. Excessive temperature and unpleasant odors are considered to be the two great causes of discomfort produced by crowded, ill-ventilated rooms.

Rüzicka[60], in 1899, called attention to a fact previously suggested by Haldane and Smith,[26] and by Rauer,[51] that insufficient tightness of the apparatus in the last of the series of cages in the Brown-Séquard and D'Arsonval ventilation experiment influences the CO_2 and thus the health of the animal in the last cage. He thought that this defect was either in the gasometer or in the cage and its connections. To determine this point, a ventilation experiment was repeated and the series so arranged that not only was the gasometer perfectly air-tight, but also the flasks and its connecting tubes. The result of experiments in which the maximum ventilation was 9.5 liters per hour and in the minimum ventilation 2.3 liters per hour, was that in the last (the fifth) cage the CO_2 content in the highest case was 13 per cent., in the lowest, 4.1 per cent. The animals present in the last cages in the majority of experiments showed no symptoms of sickness. Only in two cases were there symptoms of difficult and retarded respiration and decreased sensibility in the mouse in the last cage, and less marked symptoms in a mouse in the preceding cage.

In one experiment the last mouse soon had dyspnea and lost its appetite. Twenty-eight hours after the beginning of the experiment, with a ventilation of 3–3.4 liters per hour and a CO_2 content of 12–13 per cent., a vessel containing 90 cc. of 0.43 per cent. hydrochloric acid was inserted between the last two cages. No improvement in the animal resulted from this arrangement, even after $45^1/_2$ hours. Rüzicka concluded from his experiments that no matter how contaminated air may become as a result of respiration it possesses no acute toxic properties having any hygienic significance.

Stimulated by Brown-Séquard and D'Arsonval's ventilation experiment, Formanek,[19] in 1900, undertook to establish the chemical nature of the hypothetical poison in the expired air. In these experiments special care was taken that the cages of the experiments should be sufficiently ventilated. The animals were always taken out of the cages at night so that they might recover, and thus overcome the objection that under certain abnormal conditions the animals live and perhaps produce abnormal substances.

In his first experiment he confined a guinea-pig in each of two glass vessels provided with a tube reaching almost to the bottom for admission of air and another ending just below the stopper for the exit of air. The two vessels were connected by means of a T tube, the common branch of which was connected with a Drechsel bottle containing 100 cc. of $N/10$ sulphuric acid. Air was drawn through the apparatus by a water pump attached to the connecting Drechsel bottle. The experiment lasted six days. The air was not measured but was estimated at about 6000 liters. The cage was cleaned twice daily to avoid excremental decomposition products. At the end of the experiment the sulphuric acid was neutralized, by titration, with $N/10$ sodium hydroxide, evaporated to a small volume and the residue, taken up in 5 cc. of water, was injected subcutaneously into a healthy guinea-pig. Shortly after the injection the guinea-pig had convulsive movements of the head and then of the whole body. When laid on its back the animal had cramps of the rear extremities. In twenty minutes after the injection it was attacked by lockjaw lasting seven minutes. Soon it had cramps, stretched out its extremities and fell into a violent tetanus. This was soon followed by scraping motions of its feet, later by lockjaw, tetanus and obisthotony, of which the animal died in a few seconds, altogether in forty-two minutes from the time of injection. The autopsy finding, with the exception of a little blood effusion in the pleura and hyperemia of the internal organs, was negative. That these phenomena were not due to the sodium sulphate formed in the neutralization, was shown by a control experiment in which an equivalent amount of sodium sulphate solution was injected. No toxic effects were observed.

In an experiment designed to show whether the toxin is absorbed by acid or by alkali, two Drechsel bottles, one containing standard sulphuric acid, the other standard sodium hydroxide, were placed after the cage containing the guinea-pigs. The neutralized acid was found to be toxic, while the neutralized alkali was not.

Other experiments were performed to ascertain the nature of the toxic base; if it was ammonia or some other base. Six guinea-pigs were confined in a large glass vessel. The entering air, measured by a gasometer, was drawn through a Drechsel bottle (A) placed before the cage and filled with 50 cc. of $N/10$ sulphuric acid to exclude the possible entrance of ammonia from the air. From this Drechsel bottle the air entered the cage and then passed out and into another Drechsel bottle (B) also containing 50 cc. of $N/10$ sulphuric acid; 1704 liters of air were drawn through a 50 cc. portion of the sulphuric acid, titrated before the experiment required 49.8 cc. of sodium hydroxide for neutralization. After the experiment the acid from flask A required 49.1 cc. $N/10$ sodium hydroxide for neutralization, while that from flask B required 39.5 cc. sodium hydroxide. The contents of flask B were then made strongly alkaline with sodium hydroxide and distilled, the escaping ammonia was caught in 50 cc. of the $N/10$ sulphuric acid. Upon titration 39.5 cc. $N/10$ sodium hydroxide were required for neutralization. Hence, ammonia alone is the toxic base since no other base can be accounted for in the titration. If ammonia or any of its salts is the toxic agent, then the injection of a quantity of ammonium salts equivalent to the quantity of $N/10$ sulphuric acid neutralized by the air from the cage should produce analogous symptoms. Such amounts were calculated, injected, and similar toxic symptoms were obtained. The salts used were the sulphate, chloride, nitrate and carbonate of ammonium.

Formanek states that comparatively large amounts of ammonia must be collected in the acid wash bottle, for if small quantities of ammonia are used the animals readily stand the injection. The results obtained by various investigators might be explained as due to the use of insufficient quantities of air or larger experimental animals; those using smaller animals got positive results.

An experiment was carried out with the purpose of greatly diminishing the amount of ammonia absorbed in the sulphuric acid wash bottle by taking extreme precautions in keeping the animals and cage clean. Two Drechsel wash bottles, each containing standard sulphuric acid, were placed before and after a glass cage in which a dog was confined.

The dog was carefully cleaned before the experiment. Observations were interrupted every four hours in order to feed the dog and allow him to perform his natural functions. After 4821 liters of air had passed through the acid in the flask at the distal end of the apparatus, the acid was neutralized by the same amount of sodium hydroxide as before the experiment, showing the absence of a base. The contents of both flasks, after neutralization with sodium hydroxide, were evaporated to a small residue and injected subcutaneously into guinea-pigs. No toxic symptoms were shown in either of them.

In the earlier experiments, after injection, very violent symptoms of poisoning with ammonium salts were noticed. In the experiments of the previous investigators, one does not find any mention of such violent phenomena after the injection of condensation of absorption fluid. Considering that the amount of ammonia passing through the cage is relatively small and that a relatively small amount was found (by Lehmann and Jessen, 10 mg. per liter) Formanek conjectures that the symptoms described in earlier experiments depend on the presence of small amounts of ammonium salts. These phenomena occur most markedly in the experiments of Brown-Séquard and D'Arsonval in which were described first acceleration, and later retardation of respiration, acceleration of heart action, paralysis of the extremities, colic and diarrhoea. Other authors, especially Merkel, describe in rats a rather frequent somnolence of short duration. If ammonium salts are the poison that caused the phenomena in the earlier investigations, then the injection of smaller doses should produce similar effects. Injection of small amounts of ammonium chloride into white rats gave the following results: In doses of 8–12 mg. somnolence was evident and lasted over a long period. The respiration was difficult. Cramps and complete paralysis of the hind extremities followed a dose of 36 mg. In some experiments the animal died of general cramps. Violent tetanic cramps set in and the animal died in a few seconds if more than 50 mg. of ammonium chloride were injected. Somewhat similar results were obtained with guinea-pigs.

Formanek's work is opposed to the findings of Brown-Séquard and D'Arsonval, who maintain that there is a poisonous substance in the expired breath other than carbonic acid. He states that at times the expired air contains ammonia which is not a product of metabolism, but rather a product of decomposition in the mouth cavity, especially in those with carious teeth, or in the respiratory tract and lungs in some diseased conditions. Formanek believes that, in the experiments by earlier investigators which show the toxicity of expired air and in which the toxicity is explained as the result of the action of an unknown organic base (alkaloid), ammonia was the substance dealt with, and that this produced the same toxic symptoms which were attributed to the unknown organic substance of basic character. He also believed that the experimental animals in the successively connected cages could die either of ammonia poisoning, if the cages were sufficiently ventilated, or through carbon dioxide poisoning, if the ventilation was insufficient, and that in some experiments, ammonia, carbon dioxide and thermal influences, were together the contributing causes.

Sanarelli and Biffi,[61] in 1902, found that pulmonary exhalations of such gaseous substances as H_2S have no direct connection with the exhalation of CO_2; however, the greater the exhalation of the H_2S, the less that of CO_2. This elimination does not seem to be dependent on any fixed rule but seems to occur every time there is an abnormal production of volatile or gaseous substances in the intestines. They believed that the volatile substances (as yet undetermined) in the pulmonary exhalations, are of intestinal origin, and that they represent various products of intestinal fermentation. As to carbon dioxide, they found that the ordinary limit value of CO_2 indicative of atmospheric pollution has but slight importance.

Gaussin,[22] in 1902, investigated the chemical and physiological properties of expired air. For the collection of condensed expired air, he exposed the trachea of a dog, 40–50 kilograms in weight, and into the incision introduced a canula bent at right angles to which the trachea was tied. This canula was connected with a double valved respiration apparatus which permitted the separation of the currents of inspired and expired air. The expired air was led from the apparatus into a glass spiral of wide calibre surrounded by ice. The condensed liquid was collected in a flask, which, as well as the other apparatus, had been sterilized or cleaned with the greatest care. The dog thus treated was tied down and left in this position in all the experiments for a day. The quantity of condensed fluid during this time varied in general from 400 to 600 grams.

The results obtained upon examination of the condensate were quite different from those of Brown-Séquard and D'Arsonval. The liquid thus obtained was always clear and odorless. The evaporation of 200 grams of this liquid at over 60° C. left no appreciable residue (about 0.001 gram). Its density was constantly near that of water. The reaction to litmus was neutral or slightly acid; the acidity was attributed to the dissolved carbonic acid. The liquid did not reduce potassium permanganate either in the cold or when heated, and had no effect upon ammoniacal silver nitrate, gold salts nor platinum salts. Neither concentrated alcohol nor Bouchardet's reagent gave a precipitate. Nessler's reagent revealed traces of ammonia but not in a constant manner.

In a first series of experiments, Gaussin studied the action of the condensed expired air when injected into different animals (dogs, rabbits, guinea-pigs) and in different routes (intravenous, subcutaneous, peritoneal). In some experiments the condensation liquid from the same animal was injected for comparison into animals of different species. In other cases, the liquid from the same origin was injected into the same species but by different routes.

In another series of experiments he investigated the physiological action of the liquid concentrated in vacuum by evaporation over sulphuric acid. Tracings of the arterial pressure (carotid artery used), respiration and time were taken. A six-kilogram dog was used. When a normal pressure had been inscribed for several minutes he injected into the femoral vein 20 cc. of liquid resulting from the concentration in vacuum of 480 cc. of normal liquid. Immediately after the injection there was noticed a retardation of respiration. The tracing also showed a slight diminution of the arterial pressure. These phenomena lasted but a few minutes. The inscription was continued for an hour and gave tracings identical with the normal tracings made in the beginning. In another experiment, 30 cc. of liquid (representing 610 cc. of normal liquid) that was concentrated in vacuum was injected into the femoral vein of a dog weighing 6 kilograms. Two hundred cc. of the concentrated liquid from the evaporation in vacuum of 1130 grams of normal liquid was injected into the jugular vein in 40 cc. portions, during a period of 15 minutes. There was no change in arterial pressure or respiration even at the end of one hour.

In a third series of experiments this investigator studied the action of portions of the liquid obtained at different stages of the distillation. The first 65 cc. that went over on distillation was collected from the vacuum of 860 grams of condensed liquid from three dogs of 38–45 kilograms. The carotid artery and jugular vein of a 7-kilogram dog were exposed and tracings of the arterial pressure and respiration taken. On injecting into the jugular vein 65 cc. of liquid (first 40 cc. then four minutes later the other 25 cc.), no change in the tracings were observed.

Gaussin concluded that the liquid obtained by condensing the water vapor and the other volatile products contained in the expired air does not possess any toxic properties. The concentrated liquid, injected in doses which represent a considerable quantity of the natural liquid, did not exhibit any toxic properties. Since, according to Brown-Séquard and D'Arsonval, the toxic substance contained in the liquid obtained by condensing the expired air is volatile, on distilling a considerable quantity of this liquid and collecting the first portions which pass over on distillation, one should obtain a liquid whose toxicity could be readily demonstrated. Such a liquid did not prove to be any more toxic than the natural liquid. Gaussin concluded that the presence in the expired air of a volatile and toxic principle cannot be recognized as the cause of discomfort in public places.

Wolpert,[73] in 1903, reported experiments on the effect of breathing expired air upon the CO_2 exchange. He found that the accumulating expired air of persons confined in a respiration calorimeter diminished the normal CO_2 exchanged from 3–5 per cent. Neither pure CO_2 nor oxygen diminution had the same effect. This author concluded that the ill effects of badly ventilated rooms were due to a decrease in the CO_2 exchange, which thereby disturbs the normal metabolism.

In 1905 a series of contributions were made by Flügge and his pupils. Heymann[34] repeated the work of Wolpert. The regular distribution of CO_2 in the experimental chamber found by Wolpert was doubted on the ground that such could not be the case from the rather quiet behavior of the subject; the difficulty of a complete exclusion of the outer air, and the methods of computation. From his own work he found that in some experiments there was a decrease in the CO_2 exchange, while in others it was either maintained at the same level or made irregular jumps. He attributed the diminution of the CO_2 exchange in the experimental chamber to the quiet behavior of the subjects and their abstinence from food, and to the increase in the temperature of the chamber during the experiment. It was found that the air immediately surrounding the head of a person has a greater CO_2 content than the rest of the room, and that people very often breathe air containing large quantities of CO_2.

Paul[46], in 1905, made a series of experiments to ascertain the effect of breathing an atmosphere contaminated with impurities from respiration, at the same time regulating the conditions of temperature and humidity. In these experiments a tight glass chamber of about three cubic meters capacity, was provided with arrangements for warming, humidifying, drying and setting in motion the enclosed air. The subjects were persons in part who belong to the leisure classes and were used to cleanliness in care of skin and mouth, and in part belonged to the laboring classes and formed a contrast to the others.

As a result of confinement for 3–4 hours in the chamber, the CO_2 content of the enclosed air was raised to 10–15 per cent. There was consequently an accumulation of CO_2 which never occurs under ordinary conditions, but as long as the temperature and humidity were kept low, the subject did not experience any ill effects. Tests were also made to determine the mental condition with a tonometer, ergograph and aesthiometer and by giving the subjects calculations to perform. All such tests showed normal as long as the temperature and humidity of the chamber were kept low. Further in a school-room in which fifty to sixty children were kept continuously for two to three hours, at a temperature below 19° C., and a humidity not exceeding 50 per cent., the unpleasant phenomena in teachers or children were entirely absent, in spite of a marked increase in the gaseous products of respiration, and at the end no signs of fatigue as shown by calculation tests could be determined.

Opposed to the above findings the chamber experiments resulted in disturbances, such as headache, oppression, etc., which are often observed in crowded and poorly ventilated places, in from ten to fifteen minutes, under the following conditions: chamber temperature 26° C., humidity 50 per cent., temperature 24°, humidity 75 per cent. and at a temperature of 21° with the chamber walls warmed from the outside. Even before the appearance of the subjective disturbances just mentioned, objective effects could be demonstrated, as shown by an increase in the temperature in the skin of the forehead. By setting the air of the chamber in motion, without altering its chemical composition, the physiological disturbances were immediately removed. The persons experimented on had a pleasant expression, as if fresh air had been supplied to them.

It follows that the appearance of physiological disturbances are consequences of increase in temperature. This was shown more clearly by experiments in which the subject was allowed to keep only his head outside the chamber, or to inspire air, through a tube, from outside the chamber. On thus breathing the fresh outdoor air no favorable effect was exerted on the discomfort and oppression experienced by the subject; relief was experienced when the chamber air was set in motion. On the other hand, if the body of the subject was placed outside the chamber, no discomfort was observed when the vitiated chamber air was inhaled through a tube, except that the bad odor was annoying.

Ercklantz[17] investigated the behavior of diseased people with the same glass chamber and technic as that described in the experiments of Paul on healthy people. Among these people were some suffering from weak hearts and chronic kidney troubles, emphysema, lung catarrh and Basedow's disease. His subjects included four scrofulous school children who were stunted in growth. In the majority of the experiments the CO_2 content of the air reached from 7–15 parts per 1000. Observations were made regarding the skin temperature and moisture of the clothing.

At a moderately increased temperature and humidity, the subjects remained free from depression, while healthy adults felt discomfort. Those sick with emphysema and kidney troubles showed only a slight reaction at a higher temperature and humidity, usually less than healthy people under the same conditions. On the other hand, those with heart disease and particularly one woman afflicted with Basedow's disease, were especially sensitive to high temperature, particularly, if accompanied by a high humidity. These experiments also showed that chemical contamination of the air, as the result of respiration, is without effect on the comfort of man, and the only factors influencing discomfort, were the humidity and temperature of the surrounding air.

Flügge,[18] in 1905, summarizes these investigations as follows:

1. "Numerous researches, with accurate experimental methods and with exact regard to thermal conditions, on

both healthy and diseased subjects, have shown that the chemical variations in the composition of the atmosphere, which occur in inhabited rooms through the gaseous excretions of men, do not exercise an injurious effect on the health of the occupants.

2. "Whenever in closed crowded rooms certain impairment of health ensues, such as headache, dizziness, nausea, etc., these symptoms are to be attributed solely to heat retention.

3. "The thermal conditions of the surrounding atmosphere, *viz.*, temperature, humidity and movement are of considerably greater importance for our welfare than the chemical composition of the air. Further the refreshing sensation noticed on abundant ventilation of closed rooms, or in the open air results not from the greater chemical purity of the air, but from the more rapid loss of heat from the body.

4. "Overheating of our living rooms must, therefore, from a practical view, be avoided. While this is often difficult to accomplish during the height of summer, it can easily be carried out during the rest of the year.

5. "First and foremost, the heating arrangements should be so regulated that the temperature never exceeds the limit of 21° C. (70° F.). Especially should this control be exercised in public rooms such as schools, etc. As a rule, the temperature of heated rooms should be between 17° to 19° C.

6. "In overheated rooms a certain relief can be brought about by artificial circulation of the air, without the introduction of air from outside.

7. "To bring about relief in heated rooms by ventilation is dangerous and should be avoided in winter during the use of the rooms by persons, since chill readily ensues from the influence of cold air currents on the overheated skin.

8. "However, overheating can easily be avoided by periodical ventilation of the rooms during the time that they are not occupied.

9. "From the unpleasant odors present in living rooms, which result chiefly from the decomposition of matter on the skin and mucuous membrane and also on the clothes of the

occupants, no injurious effect on health has been demonstrated.

10. "However, these odors cause a feeling of nausea on entering the rooms and, therefore, from a practical point of view should be removed.

11. "This can be accomplished partly by prevention and deodorization, and partly by continuous exhaust ventilation, or by periodical thorough ventilation of the rooms while unoccupied.

12. "Ventilation has nothing to do with freeing inhabited rooms from dust or contagion. I would repeat that without doubt, symptoms of ill-health are observable in crowded rooms and that a bad smelling atmosphere is certainly objectionable to those who are sensitive to it, and that very great importance must be ascribed to the enjoyment of the open air; but I would make also clear the object of these requirements. It is not the chemical composition of the air, but the overheating of rooms that has the chief evil influence on health, and for that reason it is the latter that must be combatted. The objection to a bad smelling atmosphere is to be sustained, not on account of its poisonous properties, which have never been proven to exist, but on account of the resulting feeling of nausea; and fresh air is desirable for people, not because they then breathe chemically purer air, but because the continual movement of the fresh air facilitates the loss of heat from out bodies and exercises besides a very beneficial influence on our skin."

Reynolds and Lipp,[55] in 1906, reported the results of an investigation of stable ventilation. They confined cattle in a closed stall 9 feet wide, 10 feet 8 inches long, and 8 feet 2 inches high. The windows were made as tight as possible. After the stall had been in use for a short time very little air could enter. In order to feed and water the animal in the closed stall, without opening the door, a hole 14 × 18 inches was cut in the door, about 3 feet from the floor; this was covered with a tightly fitting slide that could be raised to admit the feed. The total time the slide was open did not exceed 2 minutes per day. The CO_2 content varied all the way up to 2.67 per cent. and the relative humidity up to 99 per cent. Moisture gathered freely on ceilings and walls.

Three animals, a bull and two steers were experimented upon. It was shown that when the air was gradually contaminated the cattle acquired a very high degree of tolerance. In one case, a steer, after confinement in a closed stall for 37 days, seemed to be in perfect comfort, and continued to make true gains of 1 lb. per day during the entire period. He was taken out of the stall once a week, to be weighed, and to permit cleaning of the stall, being out each time about 60 minutes.

In another case a steer was confined in a closed stall continuously for 28 days. The ventilation this animal received was that given during the brief time when the slide was opened for feeding. There was also a slight amount of air leakage around the window casing and around the close fitting door. Yet, at the end of the period, the steer appeared to be in perfect health.

It was discovered that one of the steers' horns which became broken, healed with the same rapidity as that of a steer outside whose horn was purposely broken at the same time for the purpose of comparison.

A number of blood counts and other tests were made. The only records that materially varied in a long series of averages as between the effect of confinement of cattle in an open stall and the closed stall conditions, as stated for the closed stall, were the pulse, which was very slightly increased, the respiration slightly increased, and the average period required for blood clotting was quite materially increased.

No injurious physiological effects were detected. Reynolds and Lipp concluded that carbon dioxide in the amounts ordinarily present in poorly ventilated stables is not harmful.

Peters,[48] in 1906, reported on the action of condensed human expired air on the isolated heart of the frog. He collected the condensed water by having a person breathe into a 12-liter flask, which was surrounded by a freezing mixture. The flask was closed by a double-holed stopper, through one opening of which there passed a narrower tube for the exit of air, while through the other there passed a wider bent tube which was directly connected with a short T tube. This served as a mouth-piece and, at the same time, the descending branch served for the collection of saliva, which was thus prevented from getting into the apparatus. As the T tube was short, condensation in the tube was prevented. Ten to fifteen cc. of the fluid were collected per person per hour.

The condensation liquid was clear and odorless, neutral in reaction to litmus, and very slightly acid toward phenolphthalein. For example, using phenolphthalein, 48 cc. of condensed fluid required 0.45 cc. $Ba(OH)_2$ solution. Since 1 cc. of the $Ba(OH)_2$ solution corresponded to 1.27 mg. CO_2, 100 cc. of the condensed fluid would contain 1.2 mg. CO_2.

Peters found that the condensation water from the expired breath slightly weakens the activity of the isolated heart of the frog. He believed that this action is due to the presence in the condensed fluid of some unknown substances.

In 1907, Benedict and Milner,[4] in reporting the results of their experiments on the metabolism of matter and energy in the human body incidentally took up the question of ventilation. Their experiments were carried on in a respiration calorimeter which consisted in brief of a metal-walled chamber 7 feet long, $4^1/_2$ feet wide and 6 feet high, through which an air current was passed. Analysis of the air before it entered the chamber and after it left the chamber gave data for computing the quantity of CO_2 and water vapor eliminated by the subject. To maintain an air current sufficient to supply 3000 cubic feet of pure air per hour would require the complete change of air in the apparatus about every four minutes, which was impracticable. In some preliminary experiments it was found that persons could remain several hours in stagnant atmosphere of the air-tight chamber, with no special discomfort other than that produced by a rise in temperature. After a cooling apparatus was installed the period could be prolonged without discomfort but no experiments were made to determine just when discomfort would be experienced.

In all the tests made with the apparatus after cooling appliances were introduced no discomfort was felt at any time; consequently experiments were made to determine the effect of a small ventilation. An air pump so adjusted as to supply about 4 cubic feet of air (100 liters) per minute through the apparatus or only one-twelfth of that considered requisite. In some of the experiments this was cut down to 3 and even $2^1/_2$ cubic feet per minute. The average temperature of the air inside the chamber was practically constant at 20° C. At no time even when the experiment lasted five to nine days did the subject experience any discomfort. The analyses of the air coming from the chamber showed, under all conditions, a high degree of CO_2. In experiments where the subject was at rest, the CO_2 content was often twenty-three times the normal content or 39 parts per 10,000. In experiments in which the subject was required to do hard physical work such as riding a stationary bicycle, the CO_2 increased. One test showed the CO_2 contents to be sixty-one times as great as that of normal air, or 183 parts of CO_2 per 10,000 parts of air, and yet the subject did not complain of any ill effects. On opening the window of the chamber after an experiment, the air inside invariably felt close to an outsider, yet it was unnoticed by the subject himself. On several occasions a blood count was made before and after the experiment to determine if an anemic tendency was developed, but in all cases the results were negative. Physically and mentally the subjects were apparently entirely unaffected by the long sojourn in the vitiated air.

The air which entered the chamber was dry and the moisture generally came from the subject. The quantity of moisture in the air of the chamber was greatly reduced in the work experiments by the cooling apparatus, which consisted of a water coil through which water was circulating. To aid in the determination of the total amount of water vapor leaving the chamber, the outgoing air was similarly conducted through cans immersed in cold brine. The larger proportion of the water leaving the chamber was deposited in these cans. In the work experiments the temperature of the cooling apparatus was frequently below dew-point, and the moisture was condensed on this apparatus. During the work experiments a liter or more of water was thus condensed each day. The relative humidity during this time was about 75 per cent. and during periods of rest it was much lower.

If there were any accompanying impurities of a toxic nature in the air of the chamber, such would be condensed on the surface of the heat absorber inside the chamber or in the pipes and cans through which the outgoing air was passed. A large number of experiments were made in which quantities of the liquid thus condensed were injected subcutaneously into white rats, but in all cases the results were negative.

Benedict and Milner conclude that an increase in the amount of carbon dioxide present in the air is absolutely without effect on the mental and bodily comfort of the subjects of the experiments and further, that the respiratory exchange and heat output accompanying this increase is in no way affected.

Henriet,[28] in 1907, criticized Flügge's conclusion that the effects of vitiation of air are due only to physical factors on the ground (1) that in those climates where the temperature and humidity, mentioned by Flügge, should produce impairment of health such is not the case and, (2) that the results of analysis of the condensation water indicate the presence of volatile organic matter. Neither of these objections can be sustained; the first objection is not valid, and as to the second, so little is known regarding its effects, that any positive statement at the present time is at best a conjecture.

Henriet collected the air expired through the nostrils and condensed the water vapor in a cooling vessel. The liquid thus obtained is colorless, has an insipid odor and is neutral in reaction. When distilled with a base, the condensed vapor liberates a gas with a strong ammoniacal odor, which is alkaline and very soluble in water. If, however, it is heated with a fixed acid like sulphuric acid, on distillation, acid products, having strong reducing properties, pass over; reduction of silver nitrate occurs on boiling, while reduction of chromic acid in sulphuric acid solution occurs in the cold.

On allowing the condensation liquid to evaporate at the ordinary temperature and pressure over sulphuric acid, a residue of about 0.5 gram per liter of liquid is obtained. Examined under the microscope, the residue seems to be entirely crystalline. When warmed to 36 cc. for 24 hours, it loses 21 per cent. of its weight, but if kept at 80° C. for the same time, it loses 54 per cent. of its original weight. If re-examined under the microscope, after warming at 80° C., it is still crystalline, although it is noted that certain crystals have disappeared, leaving their impression, however. Crystalline character of the residue had been observed in 1890 by Lehmann and Jessen, who believed it to consist of calcium silicate from the glass. If now the residue is brought over a gas flame, it remains crystalline, but becomes brown very easily and only in places, which proves that non-volatile organic matter is present in very small amounts. The remainder is, therefore, of mineral origin, of which one part is soluble in water and contains chlorides, while the other insoluble, part dissolves in hydrochloric acid and contains calcium. The organic constituents render the condensed water putrescible.

Henriet believed that these organic substances, when spread over the confined air, dissolved minute droplets of moisture produced health disturbances sometimes observed in poorly ventilated crowded rooms. He called attention to the necessity for ventilation, for the maintenance of a proper humidity, which would prevent the condensation of these organic particles on the walls and tapestry of the room, and on the clothes of the occupants, and thus obviate the concomitant disagreeable odors of these substances.

In the course of a paper on the toxin obtained from fatigued muscle Weichardt,[69] in 1907, incidentally mentioned that he obtained a similar toxin from the expired breath, called by him kenotoxin. He passed the expired breath into a vessel surrounded by a freezing mixture and then evaporated the condensed liquid to dryness in a vacuum. Mice, into which a part of this residue was injected, became lethargic; their temperature sank considerably, and the breathing became slower. Control mice previously immunized with the specific antibody and then injected with another part of this residue, remained lively.

These results led Weichardt,[69] in 1908, to make a more detailed investigation of the subject. He collected the expired air, either by condensing it directly, or by passing it into a water solution. Since he obtained better results with the latter procedure he adopted that method for the collection. In this work the expired breath was passed into a vessel containing a large quantity of water, acidified with a drop of hydrochloric acid, and the mixture then concentrated under a high vacuum at 30° C. to about 2 cc. The liquid was neutralized with sodium hydroxide, dialyzed, and made isotonic. One-half of the resulting fluid was injected into a normal mouse and the other half into an equally large mouse, but one that had been previously immunized with the specific antibody of the kenotoxin. The normal mouse became more and more lethargic as the body temperature went down and the breathing became slower, while the previously immunized mouse remained quite lively. The body temperature of the normal mouse treated with kenotoxin had an after temperature of 30°, and sometimes a temperature as low as 25°, while the one protected by the antibody had a temperature of 37° C. The symptoms always disappeared the next day.

Weichardt believed the kenotoxin to be a protein cleavage product of high molecular weight, that it was invariably present in the human expired breath at least in traces and that it was in no way a strong poison. Alkaloid-like substances were never found by him in the condensation water from the expired breath. He states that the kenotoxin is very labile; that it is destroyed at a moderately high temperature and loses its efficiency in about 24 hours.

Kymograph curves of a normal mouse injected with kenotoxin showed a diminution in muscle activity, while the opposite was shown by a mouse previously immunized with antikenotoxin. Weichardt found that the CO_2 exchange is diminished in the animal treated with kenotoxin. Whereas Weichardt claimed that he obtained the toxin by passing the expired breath through dilute hydrochloric acid solution, Merkel,[43] in 1892, stated that the volatile toxic base lost its toxicity after combination with acid.

In experiments upon animals, mice or guinea pigs were confined in a glass vessel connected with an upwardly inclined tube consisting of a series of bulbs. This bulbed tube was joined through a Drechsel bottle with a gas meter. Air was aspirated through the apparatus for about 10 hours and the expired breath of the animal was drawn through an absorbing solution of dilute hydrochloric acid contained in the Drechsel bottle. A micro-burner placed under the connection between the animal cage and bulbed tubes prevented the premature condensation of water vapor from the expired air. The expired breath solution was concentrated in the usual way, the liquid boiling so briskly that in the course of a half hour 300 cc. had gone down to a few cubic centimeters. Injection of this material after the usual treatment was followed with results such as retardation of respiration, reduction of temperature, etc., similar to those previously obtained. The CO_2 exchange of a mouse injected with the kenotoxin was less than half that of a normal animal of the same size and breed, while that of a previously immunized animal on injection with kenotoxin gave values slightly higher than the normal. No relation was found between the amount of organic matter and the kenotoxin content. Weichardt concluded that with insufficient ventilation there is danger of kenotoxin poisoning which shows itself next in greater sleepiness, depression and sensitiveness.

Hough,[34] in 1910, describes an experiment in which a person was confined for an hour or more in an air-tight box 3 feet wide 5 feet long and 7 feet in height. The CO_2 content rose to 50 parts or more in 10,000. When the observer opened the door the odor of the air within was almost overpowering. Yet, if the water vapor was absorbed and the temperature kept down, the subject of the experiment had not only been unconscious of this odor, but had suffered no discomfort.

Further, the quality of the mental work performed by him was also not effected.

At Flügge's request, Inaba,[35] in 1911, repeated Weichardt's work. The latter instructed Inaba in his methods and was present during Inaba's early experiments. In this work, the breath was exhaled into a vessel containing a little physiological salt solution.

In the first four experiments, Inaba's injection results with the condensed fluid were the same as Weichardt's, that is, they resulted in a lowering of body temperature, retarding of respiration and stupor, but these symptoms were by no means constant. In further experiments, Inaba found that the subcutaneous injection of small quantities (0.3 to 0.7 cc.) of the condensed fluid resulted in either no reduction of temperature and lassitude or in a slight reduction in same; and moreover, that these phenomena occurred in a marked degree only after the injection of more than 1.5 cc. But even in such doses, the effect was not always clear, but sometimes occurred also with small quantities. It was also shown that the pre-treatment of animals with kenotoxin showed no convincing results, as against those not so treated.

These facts lead Inaba to suspect that these symptoms might be due to the dilution of the salt solution used for absorbing the condensed fluid of the breath, thus, weakening the isotonic condition of the fluid. Accordingly, control experiments were made in which distilled water and also physiological salt solution were injected into animals.

These control experiments showed conclusively that the subcutaneous injection of 1 cc. of distilled water into mice weighing between 9–13 grams produced a lowering of the body temperature, stupor and retardation of respiration, that is, the same symptoms as kenotoxin poisoning, whereas the injection of physiological salt solution gave negative results. Similar results were obtained on collecting the expired breath by condensation in a weak hydrochloric acid solution, which was then neutralized and dialyzed. One part of the solution was injected as such, and the other part made isotonic. The isotonic solution on injection into a mouse had no effect, while the non-isotonic fluid produced the same symptoms as those previously mentioned.

In another experiment the liquid was collected by having the subject breathe into a vessel containing no absorbing fluid. For this purpose Inaba used a 5-liter flask closed with a double-holed rubber stopper. Through one opening there passed a narrow glass tube for the escaping air, through the other a wider bent glass tube reaching near to the bottom. The latter tube was connected at the upper end by means of a short rubber tube with a short wide glass tube which served as a mouth piece. A loose piece of cotton placed in the rubber tubing served to keep back saliva. Persons of different ages were used. The subjects were required to clean their teeth and mouths thoroughly. The condensation water amounted to from 15–16 cc. per hour. The liquid thus collected was clear, neutral in reaction and gave a yellow color with Nessler's solution.

After concentration, the liquid was injected into mice subcutaneously either without the addition of salt solution or after the preparations of isotonicity by the addition of ten-fold physiological salt solution. The results were similar to those previously obtained.

Experiments were carried out with animals, using Weichardt's method. Similar results were obtained when the condensed liquid was injected without being made isotonic. If, however, the condensed fluid was made isotonic, the animal was unaffected.

Weichardt,[70] in 1911, took issue with Inaba upon the results reported by him. He denied the use of anisotonic solutions in his work, and stated that he always used isotonic solutions. He further took issue with Inaba on his results from the injection of distilled water. While admitting that distilled water is toxic when injected intravenously, he claimed that after subcutaneous injection of distilled water, as was done by Inaba, injury to warm-blooded animals is far less. In the case of a 10–12-gram mouse he stated that the injection of $^1/_4$ cc. of fresh distilled water brought to body temperature, causes no reduction in temperature, but on the contrary, would cause arise of about 0.5 to 1° C. Weichardt admitted that repeated injections of distilled water into mice, at intervals of a short time, produced toxic symptoms, but he attributed this result to the formation in the body of protein cleavage products which induce the characteristic phenomena of temperature fall, breath retardation and sopor.

The latter author attributed thermo-labile properties to his kenotoxin from the results of the following experiment: The respired air, so far as possible residual air from a fatigued person of over sixty years old, was exhaled for several hours, through 10 cc. of distilled water to which 0.3 cc. of normal salt solution are added, and the solution concentrated in vacuum to 2 cc., neutralized with sodium hydroxide and divided into two portions, now isotonic. On now injecting a mouse with one portion, he got symptoms of kenotoxin poisoning. On diluting the other portion to 10 cc., evaporating to dryness at boiling heat, the toxin became inactivated. This he showed by dissolving the residue in a cubic centimeter of distilled water and injecting in the same manner as the first portion, when he found that the mouse was unaffected. Contrary results were obtained by Brown-Séquard and D'Arsonval[12] who found that boiling the condensed liquid had no effect on its toxicity. Weichardt's finding in this connection is inconclusive since he performed no control experiment.

Weichardt now performed experiments to show the presence of protein cleavage products *in vitro*. The expired breath was collected as in the experiment just above mentioned, except that 10 cc. of distilled water was used instead of water with salt solution; the product was concentrated to 3 cc. in a vacuum and evaporated to dryness on a weighed watch glass. A residue of 9 mg. was obtained. The residue from the evaporation of 10 cc. of distilled water gave no visible residue. He states that the residue consists mostly of organic substances which become brown when heated on platinum foil with the generation of burnt odors. It was also noted that a very characteristic odor appeared when the expired breath water, which was acid with hydrochloric acid, was made alkaline with sodium hydroxide.

Weichardt reported finally that the blue coloration obtained in the guaiac reaction failed to appear when protein cleavage products, that is condensation material from expired air, were added to a catalytic agent like a hemoglobin solution. Expired air was passed for hours into physiological salt solution. One cc. of this solution was placed in a series of test tubes, and decreasing quantities of a strongly diluted solution of fresh blood were added, and the mixture warmed at 37° C. on the water bath. Upon the addition of 0.1 cc. of guaiac solution, a blue coloration gradually appeared in some tubes and not in others. As a control, physiological salt solution was used instead of breath water.

Weichardt applied the guaiac reaction to the condensed moisture from the air of a poorly ventilated bedroom, in which a number of people were sleeping. Petri dishes containing finely powdered calcium chloride were exposed over night to this atmosphere. The calcium chloride became deliquescent and a test of the liquid from the molten mass also inhibited the guaiac reaction.

Hill, Rowland and Walker,[32] in 1911, published results of experiments that are of unusual interest in showing the fallacy of assuming carbon dioxide to be the important toxic constituent of expired air, in relation to ventilation conditions. Heat and humidity are of far greater importance.

The tests were performed in an air-tight chamber of about 3 cm. in capacity and fitted with an electric stove, three electric fans in the roof and glass windows.

In the first series of experiments eight men were shut up in the chamber in which there was no ventilation. After 44 minutes the dry bulb stood at 87° F., the wet bulb 83° F.; the CO_2 had risen to 5.26 per cent. and the oxygen had fallen to 15.1 per cent. The discomfort felt was very great, all were wet with perspiration, and the skin was flushed. The talking and laughter of the occupants had gradually become less and finally ceased.

Immediate relief resulted after turning on the electric fans and whirling the air in the chamber—very great relief in spite of the continued rise of the temperature in the chamber. On turning off the fans the discomfort returned—the occupants cried out for the fans. It is of remarkable interest that no headaches or after effects have followed this type of experiment, which has been repeated five times.

In the second series of experiments two men were shut up in the chamber, a subject and an observer. The electric heater was used to raise the temperature to about 85° F., wet bulb. The subject inhaled through a soda-lime tin and exhaled through an air meter. In this way only traces of CO_2 were inhaled. The pulse frequency, pulmonary ventilation, and body temperatures were recorded. The action of the fans gave complete relief to the feelings of discomfort and lowered the pulse frequency. Having proved this, the subject ceased breathing through the soda-lime tin. A bag containing sufficient CO_2, to raise the percentage in the chamber to about 2 per cent., was opened and the CO_2 allowed to escape into the chamber unknown to the subject. The sudden increase of CO_2 had no influence on the discomfort; it was not increased, in spite of the increased depth of the respirations. The fans relieved the discomfort as before, and *vice versa*.

Weichardt and Stötter,[72] in 1912, studied the action of the "active" substances of expired air on catalysis. One or two guinea-pigs were placed in a 2-liter bottle which was connected with two Drechsel wash bottles placed in series. The second wash bottle was connected with a gas meter and the latter with a suction pump. The glass bottle, in which the animals were kept, was cleaned every hour. The expired air from the guinea-pigs was conducted for a definite time through a weighed quantity of washing liquid and the increase in weight determined at the end of the experiment corresponded approximately to the quantity of water taken up. The guaiac reaction was carried out with 3 cc. of this solution and 3 cc. of a control solution of the same density using 0.1 cc. of fresh guinea-pig blood as a catalyzer, after both solutions had acted on each other for fifteen minutes. Guaiac resin and turpentine oil water were used as reagents and twice distilled glycerol as the wash liquid. Glycerol was selected, since the products capable of reaction preserved their effectiveness longer in glycerol, as an absorption liquid, than in aqueous solution.

As a result of these experiments they found that at first there was an increase of the guaiac reaction which with a longer time of experiment showed an arrest of the reaction. This increasing effect reached a maximum at a definite concentration of the "active" material. The occurrence of the arresting or increasing phase depends to a great extent on the number of animals and the quantity of air used, and even then after some time the arresting reaction slackens.

On trying the effect of various substances on the guaiac reaction it was found that carbohydrates, salts and CO_2, in dilute solutions, had only a slight effect which became greater with increasing concentration. In the case of albumin cleavage products, the guaiac test was usually accentuated in a dilute solution and inhibited in a concentrated solution.

In tests where a single person breathed for a period of an hour through the usual glycerol solution, inhibition of the guaiac reaction was not observed. They obtained better results when they made use of the principle of increased surface exposure. A small glass platform, 40 cm. in height and 22 cm. wide was suspended in front of the exit shaft of the ventilator of a theater having a capacity of 2000 cm. and holding 739 people. The platform held a little staircase made of glass sticks on which glass wool was placed. Glycerol was allowed to trickle over the glass wool and from time to time the glycerol that had run down was again poured over the glass wool. Experiments carried out in this way sometimes resulted in a complete inhibition of the guaiac reaction which lasted for hours, and at other times owing to a poor attendance at the theater or under better conditions of ventilation, in only a temporary inhibition.

Weichardt and Kelber,[71] in the same year, investigated the detection of expired air by means of another catalytic agent. Colloidal osmium was used as an indicator but in addition a color test was used to make the result of the catalytic agent visible. The test is based on the fact that the toxic organic substance in the breath inhibit the formation of the blue color produced when the iodine liberated from a solution of potassium iodide by turpentine oil water, acts on a starch solution in presence of colloidal osmium. The latter acts as the oxygen carrier from the turpentine oil.

The normal appearance of the blue color indicates that the air is sufficiently pure. If the color reaction is absent or imperfect, deleterious substances are present in the expired air. In control tests the quantity of the separated and starch-bluing iodine can be determined by titration with $^1/_{1000}$ sodium thiosulphate solution. The difference between the two values gives a measure for the influence of the catalyzer.

In trying the effect of different substances as possible interfering agents in the test, they found that if a toxic protein derivative as globin is coupled with other suitable groups, *e. g.*, hematoporphyrin, the globin no longer influences the catalyzer. CO_2, illumination gas, pure carbohydrates acetic and butyric acids, dilute solutions of ammonium chloride, and sodium and potassium chlorides had no effect on the reaction, while hydrogen sulphide, mercaptans, caprylic acid and protein cleavage products such as casein peptone, or edestin peptone have an inhibiting influence.

Specimens of air for analysis were obtained with a portable apparatus consisting of an air-pump worked by a motor, accumulators to furnish the electrical power, a gas meter and wash bottles. The air was forced through the wash bottles containing glass beads and glycerol, an absorption medium, with which the catalyzer was mixed. With this apparatus they tested the air of a schoolroom. The inhibition of the reaction thus obtained corresponded to that caused when a person breathed directly into a vessel containing the glycerol absorption solution.

W. Gilman Thompson,[65] in 1912, reporting on ventilation problems in hospitals and schools calls attention to the fact that the physiological method of studying the fitness of air for respiration, such as blood-pressure tests and metabolism tests, and symptom tests, as blood tests, headache, dyspnoea, etc., have been so neglected that few reliable data are obtainable concerning them. He found that when he substituted open window ventilation for the artificial system of ventilation in a hospital, the air became better, and the patients showed improvement.

Very interesting experiments have been made lately by a number of observers who showed that in diseases such as pneumonia, pleurisy, etc., this blood pressure is markedly subnormal, whereas it promptly rises to normal when the patient is placed on a balcony or roof in the open air.

Dr. Thompson found a difference of 20–30 mm. in patients placed in the Presbyterian Hospital in New York City, under these opposite conditions. In the same patient he repeatedly measured a difference of 20 mm. on taking the patient to the open air, returning him to the ward, taking him out again, etc. In the fresh air the cyanosis disappears, delirium subsides and many other symptoms promptly improve.

In the case of a patient recently experimented upon for Dr. Thompson, the following result was obtained: The patient was left overnight in a small room with the window tightly closed. A pipe was then introduced through which the patient breathed fresh outdoor air while still remaining in the closed room. A brief supply of this air lasting only 15 minutes, made no appreciable difference, but after the window was left open for a few hours, all conditions surrounding the patient remaining otherwise the same, the rate of metabolism, as measured by the CO_2 elimination, was increased to a very striking degree.

Amoss,[1] in 1913, could not corroborate Weichardt's experiments on the inhibition of the guaiac test for blood by means of the substances retained when exhaled breath is passed through a hydrochloric acid solution or over calcium chloride. The test was found unreliable in presence of a small amount of free acid or free alkali. Attempts were made to prove the presence of some substance which inhibits the oxidation of phenolphthalin by blood in liquids obtained from the expired breath by passing the air through weak hydrochloric acid or by condensing the moisture in the breath, but all were unsuccessful. Further attempts to concentrate these liquids by evaporation under diminished pressure or by the passage of a direct current were also unsuccessful.

Experiments were made on the residues obtained from the condensed liquid of the exhaled air. For this purpose exhaled air was bubbled through 0.1 *N* HCl contained in a Drechsel bottle and the liquid evaporated to dryness on the water bath in platinum dishes. Residues of 0.0014–0.0021 gram were obtained from 30–55 cc. of the liquid collected during 4 to 5 hours.

In one case a residue of 0.0004 gram was obtained from 25 cc. of liquid collected in 4 hours. Amoss explains this low result as due to the fact, that, in this experiment the delivery tube inside the Drechsel bottle did not dip into the acid solution until some condensation had taken place. Nesslerization in one of the four evaporation tests showed the residue to consist mainly of ammonium chloride which was naturally volatile on ignition.

Contrary to the findings of Weichardt, Amoss found that the residue does not charr or blacken on ignition. However, he corroborated Weichardt in that a characteristic odor was produced on neutralizing the hydrochloric acid through which the expired air had passed.

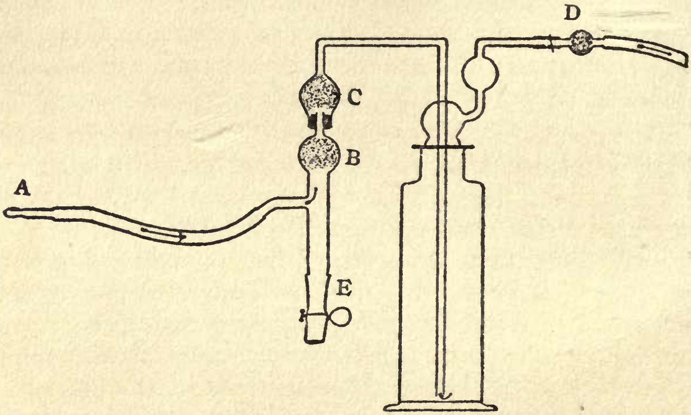
Evidence from all these experiments seems to show that the ill effect of breathing an atmosphere vitiated as the result of overcrowding in poorly ventilated places is due, not to an organic poison in the breath, but rather to the resulting physical conditions of the atmosphere, such as temperature, and humidity, and air motion. From this review of the subject it will be seen that much of our knowledge regarding the factors that produce the well-known disturbances to health resulting from a sojourn in a poorly ventilated place is still uncertain. The practical problems in connection with ventilation are also in the same unsettled condition.

In this connection it is interesting to note the following statement by Lynch in 1910:[41a] "There is nothing in ventilation of which we yet have the last word and to finalize anything about it now, is to standardize not knowledge but ignorance, and an ignorance that is colossal."

EXPERIMENTAL PART.

DESCRIPTION OF APPARATUS.

The type of apparatus for the collection of the condensations from the expired breath was the same as that used by Rosenau and Amoss. It consists of an ordinary 16 or 32 oz. Drechsel wash bottle with a bent thistle tube fused onto the main tube of the bottle. The internal diameter of the tubes is about 7 mm. A calcium chloride tube is inserted into the rubber stopper that closes the bulb of the thistle tube. On a side tube, which is fused into place about three-fourths of an inch below the bulb of the calcium chloride tube, is placed a piece of rubber tubing about 12–15 inches long, having a short piece of glass tubing about 7 mm. in diameter which serves as a mouth piece. Over the wide open end of the



A is the mouthpiece; B, C and D are glass-wool filters; E is a pinch-cock that controls the trap for removal of saliva.

calcium chloride tube is placed a piece of rubber tubing closed by a pinch-cock. This arrangement serves as a trap to catch any saliva that comes over while the subject breathes into the tube and permits its removal by opening the pinch-cock. Glass wool tightly packed in the bulbs of the thistle tube and calcium chloride tube, as well as in the bulb of a tube attached to the exit end of the bottle, serves as a filter to keep out particles and bacteria from the apparatus. The bulb tube is finally connected by means of an Erlenmeyer flask to a water pump, the suction of which helps to draw the breath thru the apparatus while the subject exhales into the mouth piece. Before use the apparatus was carefully cleaned with chromic and sulphuric acids, washed thoroughly with distilled water, dried and sterilized in an autoclave.

When the apparatus is ready for use the Drechsel bottle is kept in a freezing mixture while the two bulbs on the entrance side of the bottle are warmed by an electric bulb and kept between 37° and 40° C. so as to prevent premature condensation.

The subjects were required to wash their mouths thoroughly before beginning work in the morning and after any meal. If the trap is allowed to get filled with saliva and through carelessness the suction is interfered with, saliva is apt to be pulled over into the Drechsel bottle. It is safest, therefore, not to allow saliva to accumulate, but to remove it as soon as a little is noticed. As far as the quantity of condensation liquid is concerned, no difference was found in using either a 16 oz. or a 32 oz. Drechsel bottle. In Rosenau and Amoss' report no mention is made of the weight of the guinea-pigs used by them. In these experiments the guinea-pigs used for the injections weighed between 200 and 250 grams.

The accompanying tables give the details of the work:

Oct. 11, 1912. Liquid collected from expired breath of Weisman. Collected in accordance with the most improved method of Rosenau and Amoss as described on page 70. Time of collection $18^{1}/_{2}$ hours as follows: 9.30 A.M. to 3 P.M., $5^{1}/_{2}$ hours; 4 P.M. to 7.30 P.M., $3^{1}/_{2}$ hours; 8 P.M. to 12 M., 4 hours; 1 A.M. to 6.30 A.M., $5^{1}/_{2}$ hours. Amount collected, 27 cc. Cultures showed the liquid to be sterile. Biuret and Millon tests were negative. The condensed liquid was allowed to thaw ($^{3}/_{4}$ hour), made isotonic with ten-fold physiological salt solution and injected at once subcutaneously into guinea-pigs as follows: Guinea-pigs Nos. 1 and 2, 5 cc. each; guinea-pigs Nos. 3 and 4, $2^{1}/_{2}$ cc. each; guinea-pigs Nos. 5 and 6, 1 cc. each; guinea-pigs Nos. 7 and 8, $^{1}/_{2}$ cc. each; guinea-pigs Nos. 9 and 10, $^{1}/_{4}$ cc. each.

No. of guinea-pig.	Weight.	First injection.		Site.	Interval.	Second injection.		Site.	Result.	Remarks.
		Amt.	Date.			Date.	Amount.			
1	245	5 cc.	Oct. 12	Subcutaneous	15 days	Oct. 27	0.2 cc. normal human serum	intravenous (ext. jugular)	Tremor in 3 min.; loss of urine; normal in 10 min.	No anaphylaxis
2	248	5 cc.	Oct. 12	Subcutaneous	15 days	Oct. 27	0.2 cc. normal human serum	Intravenous (ext. jugular)	Tremor in 1 min.; hunched up; normal in 10 min.	No anaphylaxis

NOTE.—As the guinea-pigs showed no positive symptoms with injections of 5 cc., those injected with smaller amounts received no further injection.

Controls.

Blood serum control.

No.	Weight.	Date of injection.	Amount.	Site.	Result.	Remarks.
11	236	Oct. 27	0.2 cc.	Intravenous	Tremor; hunched up	Same symptoms as those following on the second injection with blood serum in G. P. Nos. 1 and 2
11*a*	225	Oct. 27	0.2 cc.	Subcutaneous	Tremor; hunched up	
11*b*	228	Oct. 27	2.0 cc.	Subcutaneous	No symptoms	Died next day. Subperitoneal hemorrhage in cecum. Slight congestion of the organs
11*c*	241	Oct. 27	2.0 cc.	Subcutaneous	Restless; normal in 5 min.	

Anaphylactic control.

		First injection.				Second injection.				
No.	Weight.	Amount.	Date.	Site.	Interval.	Date.	Amount.	Site.	Result.	Remarks.
11*d*	245	1 cc. normal human blood serum	Oct. 11	Subcutaneous	16 days	Oct. 27	0.2 cc. normal human serum	Intravenous (ext. jugular)	Difficulty in respiration; convulsive movements; died in 5 min.	Autopsy showed distended lungs; characteristic of anaphylactic shock

Oct. 16, 1912. Liquid collected from expired breath of Weisman. Apparatus same as described on page 70. Time of collection $11\frac{3}{4}$ hours, as follows: 9 A.M. to 1 P.M. = 4 hours; 2 P.M. to 6 P.M. = 4 hours; 6.45 P.M. to 10.30 P.M. = $3\frac{3}{4}$ hours. Amount collected, 16 cc. Biuret test was negative. Culture showed liquid to be sterile. 10 cc. of the liquid, made isotonic with ten-fold physiological salt solution, was injected into a guinea-pig.

		First injection.				Second injection.				
No.	Weight.	Date.	Amount.	Site.	Interval.	Date.	Amount.	Site.	Result.	Remarks.
12	246	Oct. 16	10 cc.	Subcutaneous	15 days	Oct. 31	0.2 cc. normal human blood serum	Intravenous (ext. jugular)	Tremor; hunched up	No anaphylaxis

Controls.

Blood serum control.

No.	Weight.	Date of injection.	Amount.	Site.	Result.
11*e*	228	Oct. 31	0.2 cc. normal human blood serum	Intravenous (ext. jugular)	Tremor; restless

Anaphylaxis control.

		First injection.				Second injection.				
No.	Weight.	Amount.	Date.	Site.	Interval.	Date.	Amount.	Site.	Result.	Remarks.
11*f*	241	1 cc. blood serum	Oct. 16	Subcutaneous	15 days	Oct. 31	0.2 cc. normal human blood serum	Intravenous (ext. jugular)	Jerky respirations; collapse; died in 15 min.	Autopsy showed lungs distended

Oct. 17, 1912. Liquid collected from expired breath of Weisman. Apparatus as described on page 70. Time of collection 10 hours, with interval of $1\frac{1}{4}$ hours after the fifth hour. Amount collected, 14 cc. The condensed liquid was not frozen. The Drechsel bottle was removed from the cooling vessel and allowed to stand at room temperature for a half hour. Cultures showed liquid to be sterile. The liquid was made isotonic as in previous experiments, and 10 cc. injected at once into a guinea-pig.

		First injection.				Second injection.				
No.	Weight.	Date.	Amount.	Site.	Interval.	Date.	Amount.	Site.	Result.	Remarks.
14	239	Oct. 17	10 cc.	Subcutaneous	15 days	Nov. 1	0.2 cc. normal human blood serum	Intravenous (ext. jugular vein)	Tremor; hunched up	No anaphylaxis

Controls.

Blood serum control.

No.	Weight.	Date of injection.	Amount.	Site.	Result.
11*g*	230	Nov. 1	0.2 cc. normal human blood serum	Intravenous (ext. jugular)	Tremor; loss of urine

Anaphylaxis control.

		First injection.				Second injection.				
No.	Weight.	Date.	Amount.	Site.	Interval.	Date.	Amount.	Site.	Result.	Remarks.
11*h*	242	Oct. 17	1 cc. blood serum	Subcutaneous	15 days	Nov. 1	0.2 cc. normal human blood serum	Intravenous (ext. jugular)	Expulsive respirations; convulsions; died in 20 min.	Lungs inflated, on autopsy

Nov. 4, 1912. Liquid condensed mainly from breath of Weisman; contributions were also received from six students. Apparatus same as described on page 70. Time of collection $18^1/_2$ hours, extending over a total period of 23 hours as follows:

Kubushiro	12.00 M. to 1.00 P.M.	= 1 hour	Weisman	6.15 P.M. to 7.00 P.M.	= $^3/_4$ hour	Rosenthal	8.45 A.M. to 9.30 A.M.	= $^3/_4$ hour
Weisman	1.00 P.M. to 2.00 P.M.	= 1 hour		7.15 P.M. to 9.15 P.M.	= 2 hours	Weisman	9.30 A.M. to 10.00 A.M.	= $^1/_2$ hour
	2.30 P.M. to 2.45 P.M.	= $^1/_4$ hour	Maurice	9.45 P.M. to 11.15 P.M.	= $1^1/_2$ hours	Horowitz	10.00 A.M. to 10.45 A.M.	= $^3/_4$ hour
Hirschleifer	2.45 P.M. to 3.30 P.M.	= $^3/_4$ hour	Weisman	11.45 P.M. to 3.30 A.M.	= $3^3/_4$ hours	Weisman	10.45 A.M. to 11.00 A.M.	= $^1/_4$ hour
Weisman	4.30 P.M. to 6.00 P.M.	= $1^1/_2$ hours		3.45 A.M. to 5.15 A.M.	= $1^1/_2$ hours			
Berman	6.00 P.M. to 6.15 P.M.	= $^1/_4$ hour		6.00 A.M. to 8.00 A.M.	= 2 hours			

Amount collected, 25 cc. Cultures showed liquid to be sterile. Biuret test negative. The frozen liquid was allowed to thaw (35 minutes) then made isotonic as in previous experiments, and 20 cc. were injected at once into guinea-pig No. 15.

Nov. 8, 1912. Liquid condensed mainly from breath of Weisman; contributions were also received from three students. Collection of the condensed fluid was carried out during a period of $22^1/_4$ hours, of which $17^1/_2$ hours were given to the actual collection as follows:

Weisman	5.00 P.M. to 9.00 P.M.	= 4 hours	Weisman	9.00 A.M. to 10.00 A.M.	= 1 hour	Horowitz	2.00 P.M. to 2.45 P.M.	= $^3/_4$ hour
	10.00 P.M. to 12.00 M.	= 2 hours	Maurice	10.00 A.M. to 12.00 A.M.	= 2 hours	Weisman	2.45 P.M. to 3.15 P.M.	= $^1/_2$ hour
	1.00 A.M. to 5.30 A.M.	= $4^1/_2$ hours	Weisman	12.45 P.M. to 1.45 P.M.	= 1 hour			
	6.30 A.M. to 8.00 A.M.	= $1^1/_2$ hours	Tracy	1.45 P.M. to 2.00 P.M.	= $^1/_4$ hour			

Amount collected, 19 cc. The liquid was kept frozen most of the time; at the end it was allowed to thaw ($^3/_4$ hour) and made isotonic as usual. Cultures showed liquid to be sterile. 18.5 cc. of the isotonic fluid were injected at once into guinea-pig No. 16.

		First injection.				Second injection				
No.	Weight.	Date.	Amount.	Site.	Interval.	Date.	Amount.	Site.	Result.	Remarks.
15	207	Nov. 5	20 cc.	Subcutaneous	26 days	Dec. 1	0.5 cc. normal human serum	Intravenous (ext. jugular)	Tremor	No anaphylaxis
16	202	Nov. 9	18.5 cc.	Subcutaneous	22 days	Dec. 1	0.5 cc. normal human serum	Intravenous (ext. jugular)	Tremor; hunching up	No anaphylaxis

Controls.

Blood serum control.

No.	Wt.	Date of injection.	Amount.	Site.	Result.
17	234	Dec. 1	0.5 cc. normal human blood serum	Intravenous	No symptoms
18	241	Dec. 1	0.5 cc. normal human blood serum	Intravenous	Tremor

Anaphylaxis control.

		First injection.				Second injection.				
No.	Wt.	Date.	Amount.	Site.	Intervals.	Date.	Amount.	Site.	Result.	Remarks.
19	247	Nov. 5	1 cc. normal human blood serum	Subcutaneous	26 days	Dec. 1	0.5 cc. normal human serum	Intravenous	Spasmodic respirations; cough; paralysis; death in 6 min.	Autopsy showed distended lungs.

Dec. 20, 1912. Liquid collected from expired breath of four women. Separate Drechsel bottles used for each. Apparatus same as described on pag 70.

Name of subject.	Age.	Amount collected.
1. Alice Murray	33	21 cc.
2. Mary Murray (colored)	25	24 cc.
3. Mary Philbin	30	20 cc.
4. Annie Cahon	42	30 cc.

Time of collection: all exhaled from 8.30 A.M. to 12.30 A.M. = 4 hours, and from 1.15 P.M. to 5.45 P.M. = 4½ hours; subjects 1 and 2 from 6.15 P.M. to 8.15 P.M. = 2 hours. Amount collected, 91 cc. The frozen liquids were thawed (½ hour). Cultures from each liquid remained sterile. The liquids were combined. Biuret and Millon tests negative. The mixture was made isotonic as in previous experiments, and 20 cc. were injected at once into guinea-pig No. 20.

Dec. 26, 1912. Liquid collected from the expired breath of three women. Separate Drechsel bottles used for each. Time of collection 8¾ hours, with an interval of one hour after the fourth hour. Amount collected, 66 cc. Biuret test, negative. The bottles were kept at 0° C. for three hours, and allowed to come to room temperature. Cultures from the liquids remained sterile. The mixture of the liquids was made isotonic as in previous experiments and 20 cc. were injected at once into guinea-pig No. 24.

Name of subject.	Age.	Amount collected.
1. Mary Philbin	30	18 cc.
2. Annie Cahon	42	26 cc.
3. Mary Murray (colored)	25	22 cc.

		First injection.				Second injection.				
No.	Wt.	Date.	Amount.	Site.	Interval.	Date.	Amount.	Site.	Result.	Remarks.
20	240	Dec. 20, 1912	20 cc.	Subcutaneous	31 days	Jan. 20, 1913	0.4 cc. normal human blood serum	Intravenous (ext. jugular)	No symptoms	No anaphylaxis
24	232	Dec. 26, 1912	20 cc.	Subcutaneous	24 days	Jan. 19, 1913	0.4 cc. normal human blood serum	Intravenous (ext. jugular)	Tremor	No anaphylaxis

Controls.

Blood serum control.

No.	Wt.	Date of injection.	Amount.	Site.	Result.
22	218	Jan. 19	0.5 cc. normal human blood serum	Intravenous (external jugular)	Tremor

Dec. 30, 1912. Liquid collected from the expired breath of four women. Separate Drechsel bottles used for each. Apparatus same as described on page 70.

Name of subject.	Age.	Amount collected.
1. Annie Leonard	44	16 cc.
2. Mary Murray	25	17 cc.
3. Annie Healey	38	25 cc.
4. Lizzie Greer	27	32 cc.

Time of collection: $8^1/_2$ hours with an interval of an hour after the fourth hour. Amount collected, 90 cc. The liquids were not frozen. Cultures from each liquid remained sterile. The combined liquids gave negative Biuret and Millon tests. The mixture was made isotonic as in previous experiments and 20 cc. injected at once into guinea-pigs Nos. 27, 28, 29.

		First injection.				Second injection.				
No.	Weight.	Date.	Amount.	Site.	Interval.	Date.	Amount.	Site.	Result.	Remarks.
27	239	Dec. 30, 1912	20 cc. condensed expired air	Subcutaneous	21 days	Jan. 20, 1913	0.4 cc. normal human blood serum	Intravenous (ext. jugular)	Tremor	No anaphylaxis
28	215	Dec. 30, 1912	20 cc. condensed expired air	Subcutaneous	20 days	Jan. 19, 1913	0.4 cc. normal human blood serum	Intravenous (ext. jugular)	Hunched; tremor	No anaphylaxis
29	232	Dec. 30, 1912	20 cc. condensed expired air	Subcutaneous	21 days	Jan. 20, 1913	0.4 cc. normal human blood serum	Intracranial	Tremor	No anaphylaxis

Control.

Blood serum control.

No.	Weight.	Date of injection.	Amount.	Site.	Result.
30	245	Jan. 20, 1913	0.4 cc. normal human blood serum	Intravenous (ext. jugular)	Tremor

Expired Air controls.

Jan. 6, 1913. Liquid condensed from breath of four women. Separate Drechsel bottles used for each. Apparatus same as described on page 70.

Name of subject.	Age.	Amount collected.
1. Stella Brihms	40	17 cc.
2. Mary Wiggins	35	23 cc.
3. Mary Carroll	45	25 cc.
4. Nora Byrnes	50	21 cc.

Time of collection: $8^{1}/_{2}$ hours. with an interval of one after the fourth hour. Amount collected, 86 cc. The liquids were not frozen. Cultures from the mixture of the liquids from the four bottles remained sterile Biuret test was negative. The liquids before being mixed were kept separately at 0° C. for 2 hours, then allowed to stand at room temperature (15° C.) for 20 minutes. The mixture was made isotonic as previously and injected at once subcutaneously into guinea-pigs Nos. 31, 32, 33, 36.

Feb. 5, 1913. Liquid condensed from breath of four women. Separate Drechsel bottles used for each.

Name of subject.	Age.	Amount. collected.
1. Josephine Drake	23	$10^{1}/_{2}$ cc.
2. Mary Ryan	39	6 cc.
3. Annie Leonard	44	$11^{1}/_{2}$ cc.
4. Annie Cahon	35	13 cc.

Time of collection: the first two subjects exhaled for 7 hours, resting for 1 hour after the first $2^{1}/_{2}$ hours; the other two subjects exhaled consecutively for $5^{1}/_{4}$ hours. Amount collected, 41 cc. The liquids were not frozen. Cultures showed liquids to be sterile. The liquids were combined, the mixture was made isotonic as previously, and injected at once intravenously into the guinea-pigs above mentioned.

		First injection.				Second injection.				
No.	Weight.	Date.	Amount.	Site.	Interval.	Date.	Amount.	Site.	Result.	Remarks.
31	248	Jan. 6, 1913	20 cc. condensed expired air	Subcutaneous	30 days	Feb. 5	2 cc. condensed expired air	Intravenous (ext. jugular)	No symptoms, except hunching up	No anaphylaxis
32	237	Jan. 6, 1913	20 cc. condensed expired air	Subcutaneous	30 days	Feb. 5	2 cc. condensed expired air	Intravenous (ext. jugular)	Shivering	No anaphylaxis
33	215	Jan. 6, 1913	20 cc. condensed expired air	Subcutaneous	30 days	Feb. 5	3 cc. condensed expired air	Intravenous (ext. jugular)	No symptoms, except hunching up	No anaphylaxis
36	222	Jan. 6, 1913	20 cc. condensed expired air	Subcutaneous	30 days	Feb. 5	3 cc. condensed expired air	Intravenous (ext. jugular)	No symptoms, except hunching up	No anaphylaxis

Expired Air alone.

No.	Weight.	Date of injection.	Amount.	Site.	Result.
38	214	Feb. 5, 1913	3 cc. condensed expired air	Intravenous (ext. jugular)	Shivering
39	230	Feb. 5, 1913	3 cc. condensed expired air	Intravenous (ext. jugular)	No symptoms except hunching up

EFFECT ON MAN OF INJECTION OF CONDENSED LIQUID FROM HUMAN EXPIRED AIR.

No record has been found in the literature of any experiments on the effect of injection of condensed expired air on man. Using the same apparatus for collecting the condensed expired air as described on page 70, in the course of 7½ hours 11 cc. were collected from one woman (age 40) and 13½ from another woman (age 44). Culture media inoculated with the liquid remained sterile. The liquids were mixed made isotonic with ten-fold physiological salt solution, and kept in a freezing mixture for 3 hours. The frozen liquid was then removed, allowed to come to room temperature, and immediately injected into the median basilic vein of two persons: 5 cc. were injected into myself, and 8 cc. into a pulmonary asthmatic patient 30 years old. There were no immediate or after effects of any kind that could be observed.

DOG LUNG EXPERIMENTS.

In view of the statement by Rosenau and Amoss that there is a volatile protein in the expired breath, it was thought advisable to test this matter from another standpoint. In a series of experiments the breathing process *in vivo* was simulated. In brief, the method consisted in drawing air over the macerated lungs of a dog and condensing the vapors into a Drechsel bottle standing in a freezing mixture. This condensation liquid was used as a sensitizing medium by injection into guinea-pigs. After a suitable incubation period, dog-blood serum was injected into the same animals. The animals did not react.

In these experiments the dogs were killed by bleeding and the blood collected and defibrinated. The lungs were removed immediately and ground in a mortar with sand; in some cases some of the defibrinated blood alone was triturated with the lung, and in other cases, for better extraction, blood and physiological salt solution were used.

The apparatus consisted of the following train: (1) A Woulf wash bottle containing concentrated sulphuric acid.

DOG LUNG

The lungs of a dog, weighing 10 lbs., were removed immediately after bleeding the animal. They placed in the bottle referred to as (4) in the train of apparatus. More of the defibrinated serum hours, 23 cc. of condensed fluid were collected. Cultures from the liquid remained sterile. From this origin. Ten cc. were injected subcutaneously into guinea pig No. 50. Treatment of the other

			First injection.	
	No.	Wt.	Date.	Amount.
	50	222	Jan. 22, '13	10 cc.
Dog weighed 16 lbs. Lung macerated as above with a little serum and physiological salt solution. Used 30 cc. blood serum and 30 cc. salt solution; 25 cc. were condensed in 8 hours.	48	237	Jan. 29, '13	10 cc.
Dog weighed 12 lbs. Treatment of lung same as above, excepting that 25 cc. of blood serum and 25 cc. of the salt solution were used. 31 cc. were condensed in 8 hours and 50 minutes.	53	250	Feb. 12, '13	15 cc.

Dog-blood serum

No.	Weight.	Date of injection.	Amount.	Site.
41	217	Jan. 22, 1913	0.5 cc. dog-blood serum	Intravenous (ext. jugular)
42	240	Feb. 12, 1913	0.3 cc. dog-blood serum	Intravenous (ext. jugular)
43	215	Jan. 22, 1913	0.15 cc. dog-blood serum	Intravenous (ext. jugular)
45	210	Jan. 29, 1913	0.15 cc. dog-blood serum	Intravenous (ext. jugular)
46	208	Feb. 12, 1913	0.5 cc. dog-blood serum	Intravenous (ext. jugular)

Anaphylaxis

						Second
No.	Weight.	Date of injection.	Amount.	Site.	Interval.	Date.
43	215	Jan. 22	0.15 cc. dog-blood serum	Intravenous	21 days	Feb. 12

*Guinea-pig 43, which had given negative results in the above-mentioned blood-serum control, was directly available, because of its sensitization, for this anaphylactic test.

EXPERIMENTS.

were triturated in a mortar with sand and a little defibrinated serum of the dog. The mixture was was added; the total amount used was 20 cc. Air was drawn through the apparatus. After 8 point on, the treatment of the condensed fluid was the same as in the case of the fluid of human lungs is indicated below.

		Second injection.				
Site.	Interval.	Date.	Amount.	Site.	Result.	Remarks.
Subcutaneous	21 days	Feb. 12, '13	0.1 cc. fresh dog-blood serum	Intravenous (ext. jugular)	Tremor	No anaphylaxis
Subcutaneous	14 days	Feb. 12, '13	0.1 cc. fresh dog-blood serum	Intravenous (ext. jugular)	Tremor	No anaphylaxis
Subcutaneous	23 days	Mar. 7, '13	0.1 cc. fresh dog-blood serum	Intravenous (ext. jugular)	Tremor; hunched up	No anaphylaxis

toxicity controls.

Result.	Remarks.
Shivering in 1 minute; collapse; convulsive movements of legs; dead in 10 minutes	Autopsy showed cyanotic hue of subcutaneous and muscular tissues; lungs slightly inflated.
Weak in hind legs; up in 5 minutes	
Tremor	
Tremor; hunched up	
Weak in hind legs	Congestion of intestines shown on autopsy.
After 5 hours ran around in circle; died in 6 hours	

control.*

injection.			
Amount.	Site.	Result.	Remarks.
0.1 cc. dog-blood serum	Intravenous (ext. jugular)	Jerky respirations; paralysis; dead in 15 minutes	On autopsy, lungs found distended

(2) A soda-lime tower. (3) A bottle containing 300 cc. of distilled water. (4) A wide-mouthed liter bottle containing the macerated lung with the extractive fluid. A stirring rod, with a bundle of long twisted strips of filter paper attached to it, passed through the rubber stopper of this bottle. (5) A Drechsel wash bottle with the saliva trap attached on one side of it and a bulb tube on the other. All the bulbs were packed tight with glass wool. (6) An empty Erlenmeyer flask. (7) A water pump.

Air drawn through the apparatus by means of the water pump was freed from organic matter and CO_2 by the sulphuric acid and the soda-lime. Evaporation of water from the wash bottle served to compensate for evaporation from the bottle containing the lung, thus preventing the latter from getting dry. Both these bottles were kept in pails of water, the first warmed to 40°–42° C., the second to 37°–40° C. The bulb with glass wool on the saliva side was warmed by an electric bulb to 37°–40° and the Drechsel bottle, receiving the condensations, was kept in a freezing mixture. The filter paper in the bottle with the lung served to give a wide surface and simulated in a way the living lung. Occasionally the handle of the stirring rod projecting above the rubber stopper was turned so as to stir up the contents.

The condensation liquid thus obtained was made isotonic with ten-fold physiological salt solution and immediately injected into guinea-pigs subcutaneously in amounts of from 10–15 cc. After an incubation period of 14 to 23 days, intravenous injection, by way of the external jugular vein, of 0.1 cc. of fresh dog-blood serum failed to evoke an anaphylactic reaction. Controls for toxicity of dog-blood serum were made. 0.5 cc. injected intravenously killed one guinea-pig in 10 minutes, another in 6 hours. The amount used in the injection experiment, 0.1 cc., was not toxic.

As no anaphylactic reaction was obtained, it can be concluded that no sensitizing material was present in the condensation liquid. At any rate, there is no volatile protein in lung.

PEPTONE EXPERIMENT.

As peptone gives a very sharp biuret reaction and is a representative of simple proteins, it was selected as suitable for a volatility test. Five grams of Witte peptone were placed in a flask and heated with 100 cc. of distilled water. The mixture was well shaken and allowed to stand for about half an hour. The yellow liquid was filtered and 80 cc. of the filtrate placed in a 32 oz. Drechsel bottle. The exit tube of this bottle was connected by means of a short glass tube which passed through the rubber stopper, fitting into the thistle bulb of another Drechsel bottle of the same size. The first bottle was set in a pail of water warmed between 37°–40° C., the entrance bulb of the second bottle was warmed to the same temperature by an electric bulb, while the second Drechsel bottle was surrounded by a freezing mixture. This bottle was connected by means of an empty Erlenmeyer flask to a water pump. Air was drawn through the apparatus and, in the course of 7 hours, 9 cc. of a clear colorless liquid were collected. The biuret test was negative, as were also the Millon and xanthoproteic reactions.

CHEMICAL TESTS.

Chemical examination of the liquids obtained from the condensed expired breath of several persons gave the following result:

Appearance and color: clear and colorless.

Odor: none. On allowing the liquid to stand in an incubator for three days at 37° C. and examining after intervals of 24 hours, no odor was developed. Reaction toward litmus, phenolphthalein and congo red, negative.

On evaporating 40 cc. under diminished pressure down to 10 cc. and then testing with the biuret and Millon reagents, negative results were obtained. The first 5 cc. of distillate from the evaporation of 40 cc. under diminished pressure gave a negative biuret test, the second 5 cc. gave a negative Millon test.

The following tests were negative: Sulphur, phosphorus, bromide, iodide, amine, acetone (iodoform test), diacetic acid (Lipliawski test), and protein.

Ammonia was present (Nessler reagent), also traces of chloride.

DISCUSSION OF RESULTS.

In all, eleven repetitions of Rosenau and Amoss' experiments were made, the difference from their procedure being that the condensation liquid was made isotonic with ten-fold physiological salt solution; the injection fluid was never warmed to 37° C. but injected either cold or at room temperature, and the second injection was made intravenously (external jugular vein). In one instance the second injection was made intercranially at a point on the top of the head about midway between the eyes. When it was found that 10 cc. of the condensation material failed to produce sensitization, the quantity was increased to 20 cc. and the mixed condensations from three or four persons (females) were used.

Negative results were obtained in all cases. Control experiments, using expired-air condensations for the second injection as well as for the preliminary sensitization, also failed to produce anaphylaxis.

Rosenau and Amoss record the development of sloughs at the site of the subcutaneous injections of the condensations from the breath. In no case did we observe such an occurrence. The cases observed by them were probably due to lack of isotonicity of the injected fluid.

On examining Rosenau and Amoss' work it is at once evident that no proper controls were carried out. In the first place, their control experiments for toxicity of blood serum were inadequate. In our control experiments the same symptoms (shivering, hunching up) were observed after injection of blood serum alone, as were noted after the second injection with blood serum, following a preliminary sensitization-injection with the expired-air condensation. In our control experiment for anaphylaxis fresh normal blood serum was injected into guinea-pigs previously sensitized with human-blood serum. The great weakness, however, in Rosenau and Amoss' controls is the fact that they failed to conduct expired-air-condensation controls, that is, to make the second injection as well as the first, with the expired air condensation.

As the site for the second injection, Rosenau and Amoss selected the heart or the brain (right optic foramen). They state that with a little practice, the operation on the heart of the guinea-pig is easily performed and unattended by any ill effects. There are, however, strong objections to the heart as a site of injection: (1) There is no assurance that the entire amount of injected fluid goes into the heart. (2) There may be produced (*a*) lesions of the heart or lungs, (*b*) injury to the intra-ventricular septum, (*c*) injury to the sinus venosus, (*d*) pericardial injury and hemorrhage. (3) In injury to the bundle of His in the heart tissue, symptoms may be produced like the Stokes-Adams syndrome, with resulting difficulty of respiration which may be taken for a result of anaphylaxis. And as to the injections into the brain, Rosenau and Amoss themselves state: "When the second injection was placed under the dura through the optic foramen the results were sometimes clouded by the appearance of symptoms which were interpreted to be the result of central irritation."

Another weak point in Rosenau and Amoss' work is the comparatively small number of positive reactions they obtained. Of ninety-nine guinea-pigs only twenty-six reacted anaphylactically, and of these twenty-six, but four cases were severe enough to cause death. Of the autopsy examinations, only one showed the anaphylactic picture of distended lungs.

Rosenau and Amoss stated that they obtained a greater percentage of reactions in the guinea-pigs injected with the liquid condensed from the expired breath of females than those injected with liquid condensed from the expired breath of males, and they raise the question whether this is a coincidence or not. Compilation of figures from their own tables show the following facts: In all, four men were used as subjects of the experiments and 14 positive cases of anaphylaxis are recorded. In the case of women, three subjects were used and the condensed liquid of one woman was found to be inactive. The number of positive cases of anaphylaxis recorded for the two other women was 12. From an examination of these figures it is evident that any conclusion drawn from such insufficient data is entirely without foundation.

In connection with the suggested greater toxicity of the breath of females a series of experiments were carried out in which eleven women were subjects. Injections were made using a mixture of the combined condensations from the expired breath of three or four women. Anaphylactic reactions in all cases were negative. We had previously obtained negative results with the breath from males. There was, therefore, no reason for assuming that the breath of females is more toxic than that of males.

It is interesting to note that the direct injection into the median basilic vein of two persons, of a mixture of the condensed expired breath of two women, was without any effect. One of the persons receiving the injection was a patient suffering from pulmonary asthma.

In connection with Rosenau and Amoss' statement as to the volatility of the protein in the expired breath, it is interesting to follow what they call their argument, for the further they go the less definite and logical they become. Their statement is as follows: "At first sight it seems almost incredible to believe that such a complex molecule as protein may be volatile. Nevertheless, such appears to be the natural conclusion to draw from the results of our experiments. After all, the question of volatility may have much similarity to the question of solubility. Theoretically, all substances are soluble, although some in minute amounts; in the same sense all substances may be volatile. Volatility does not mean necessarily a change to the gaseous state in the sense that simple substances are volatile. Thus we may assume that solid and liquid substances may pass into the air in a state of 'colloidal suspension.' The simplest conception would be to regard the protein as passing off in solution in the watery vapor. Whatever the physico-chemical conception the inference is forced upon us that protein may pass into the air in the expired breath and be again collected in sufficient amounts to produce a definite biological reaction in susceptible animals."

The authors admit that it seems incredible that such complex molecules as those of protein may be volatile and in defence of volatility show that they have a wrong conception of volatility when they say "volatility does not mean necessarily a change to the gaseous state in the sense that simple substances are volatile." Again the statement that "solid and liquid substances may pass into the air in a state of colloidal suspension," is an admission that the substance may be carried over *mechanically.* But if there is no change of state to the gaseous form, the substance is not volatile. Again the statement that the "simplest conception would be to regard the protein as passing off in solution in the watery vapor" is a substitution of the idea of solution for their idea of volatility. Finally, the statement that "whatever the physico-chemical conception the inference is forced upon us that protein may pass into the expired breath and be again collected in sufficient amounts to produce definite biological reactions in susceptible animals," is an assertion that, whether the reader may or may not take their explanation, it remains a fact, according to them, that "volatile protein" is present in the breath.

Rosenau and Amoss explain the sudden cases of death in adults following a first injection of horse serum (diphtheria anti-toxin) as being the result of prior sensitization with horse protein which entered the system because those persons came in close proximity with horses. In this connection it is interesting to note the following statement made by Dr. Leonard Hill[31] in the course of his vice-presidential address before the Section of Physiology of the British Association for the advancement of Science, in September, 1912: "Flax and I have for fourteen weeks kept guinea-pigs and rats confined together in a box and poorly ventilated, so that they breathed air containing 0.5 to 1.0 per cent. of carbon dioxide. The guinea-pigs proved wholly free from anaphylactic shock on injecting rat serum. Therefore, they were not sensitized by breathing the exhaled breath of the rats for many weeks, and we are certain that no foreign protein is absorbed in this way. It has been proved by others and by us that animals so confined do well, so long as they are well fed and their cages are kept clean, light, cool and dry. It is wholly untrue that they are poisoned by breathing each other's breath. The only danger arises from droplet contagion in cases of infective disease."

Feeling satisfied that they had demonstrated the presence of a volatile sensitizing substance (protein) in the expired breath, Rosenau and Amoss then state that evidently other factors besides temperature and humidity are to be taken into account in explaining the ill effects of vitiated air, meaning that among those other factors, "volatile protein" must be considered.

In our work, in which a real control was carried out by making both the final and the initial injections with condensations from expired air, no anaphylactic reactions were obtained. We can, therefore, conclude that in considering the factors other than temperature and humidity, that are possibly responsible for the ill effects of vitiated air in poorly ventilated, overcrowded places, a hypothetical sensitizing substance in the breath cannot be considered as one of those factors. Even if such sensitizing substance were present, it would have no sanitary significance except for stable men, etc., under special conditions. In the first place, the human species cannot be sensitized with the breath from the same species. In the second place, as to the direct effect of this substance if present, the amount would be so infinitesimal that its action, if any, would be negligible. Crowder,[15] in 1913, in commenting on this matter, writes: "In the air of the lungs any such volatile protein would be constantly present in fifty or one hundred times greater concentration than in the air of occupied rooms, since the air of such rooms does not ordinarily contain more than 1 or 2 per cent. of expired air. The air which has been expelled from the alveoli and has lodged in the upper air passages during expiration, is drawn back into the alveoli during inspiration. The volume of this "dead space" amounts to about one-third of the whole volume of normal quiet inspiration; consequently, the normally inspired air is approximately one-third expiration. The reinspiration of expired air appears to be a conservative and necessary process. It is a well-known fact that the pulmonary ventilation is increased and decreased reflexly to maintain a relatively constant pressure of CO_2 (about 5 per cent. of an atmosphere) in the alveolar air. Our hypothetical poison could not become concentrated in the surrounding air, unless the CO_2 also became concentrated, and then the larger respiratory ventilation due to the increased CO_2 would affect the concentration of both substances in the alveolar air in equal proportion. The increase in pulmonary ventilation which would be made necessary by the presence of 2 or 3 per cent. of expired air in that which is being breathed, in order to maintain what we must, in the nature of things, look upon as the normal dilution of the alveolar air, is very small. It amounts to no more than three or four per cent., whereas the possible increase is as many hundred per cent. Such an increase ought to lie well within our 'margin of safety.'"

SUMMARY AND CONCLUSIONS.

1. Subcutaneous injection into guinea-pigs of isotonic condensation liquid from human breath, in amounts up to 20 cc. followed by intravenous injection of human blood serum in amounts up to 0.5 cc., with a suitable incubation period between both injections, does not result in anaphylactic shock.

2. Subcutaneous injection into guinea-pigs of isotonic condensation liquid from human breath, followed by intravenous injection of the same kind of material in amounts up to 3 cc., with a suitable incubation period between both injections, is not followed by anaphylactic shock.

3. Subcutaneous injection into guinea-pigs of isotonic condensation liquid from the human breath is not toxic.

4. Intravenous injection into guinea-pigs of 3 cc. of isotonic condensation liquid from human breath is not toxic.

5. Intravenous injection (median basilic vein) into human beings of isotonic condensation liquid from human breath, in amounts up to 8 cc., is not toxic.

6. There is no evidence to show that the condensation liquid obtained from males is more toxic than that from females.

7. There is no evidence to show that proteins are volatile.

8. Whereas the anaphylactic test reveals the presence of extremely minute quantities of sensitizing material so infinitesimal that chemical tests do not respond, and whereas this test failed to show the presence of any sensitizing material in human breath, it can be concluded that so far as danger to health from ill effects of poor ventilation is concerned, the presence of sensitizing material in the breath is not a factor to which such ill effects may be attributed.

9. The results of these experiments disprove the statements of Rosenau and Amoss that the breath contains "volatile" protein and that such "volatile" protein is an important respiratory factor.

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BIOGRAPHICAL.

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A Further Study of the Bardach Test for Protein. *Biochemical Bulletin* (1912), **1**, 538.

On the Question of Protein in Expired Air. *Biochemical Bulletin* (1913), **2**, 295.

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